ASSESSING GULF COAST TICK, *AMBLYOMMA MACULATUM* KOCH (ACARI:IXODIDAE) POPULATION CHANGES IN TEXAS USING THE U.S. DROUGHT MONITOR CLASSIFICATION SCHEME

A Thesis

by

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ABSTRACT

Desiccation is the greatest risk to maximum survivorship during the off-host phase of ixodid tick (Acari: Ixodidae) life cycles. Ixodid tick development, survivorship and population responses have typically been assessed across habitat types in association with microclimate and mesoclimate temperature, precipitation, relative humidity, dew point or vapor pressure deficits, while assessment of ixodid tick populations at larger spatial scales have relied more upon macroclimate temperature and precipitation variables. We conducted a retrospective, observational study to assess *Amblyomma maculatum* Koch (Acari:Ixodidae) population changes using tick collection records and regional drought data for the state of Texas for the period 2000 – 2014.

Collection records containing *A. maculatum* were obtained from the Texas Animal Health Commission (TAHC) as submitted by inspectors at local county livestock markets. A "collection" was a single laboratory submission of one or more *A. maculatum* ticks. These records were assumed to be representative of *A. maculatum* abundance over time for each year in a 148 county study area. The Drought Mitigation Center, Lincoln, Nebraska provided Texas drought data from the U.S. Drought Monitor (USDM) for the same period and county area, based on their D0-D4 drought stress categories.

Repeated measures analyses were used to compare changes in *A. maculatum* collections and drought stress across the 15-year period between two adjacent

geographical areas of Texas, defined as Coastal and Inland zones. Data were then combined, to test whether trends in *A. maculatum* population changes could be explained by corresponding changes in drought stress.

These analyses indicated significant difference (P = <0.0001) between the Coastal and Inland zone for tick collections, from June – November annually. There was significant interaction (P = <0.0001) between year and location for all drought stress categories. Subsequently, the combination of drought stress categories (D2-D4), across a 2-year lag was significant for both January – December and June – November tick collections, P = 0.0029 and P = 0.0043, respectfully.

These results support our hypothesis that *A. maculatum* population changes in Texas can be associated with drought stress levels of the USDM and a 2-year rolling predictive model is feasible

DEDICATION

I dedicate this project to my husband, Thommy McGowan-White, without whose support this would not be possible. I am humbled and grateful for the sacrifices he has made in his own career and higher educational pursuits so that I could pursue mine. Even as team players it can be hard to take a seat on the sideline while others are knee deep in the fray, but now it's time to switch places! From Korea, to Texas, to Charleston, SC, to Aggieland, who would have guessed twenty-two years ago that this would be our path? I still *can't believe you actually picked me*....but with you beside me, hand in hand anything is possible as we embark upon the next chapter together, Love Always CSP.

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NOMENCLATURE

ACH	American Canine Hepatozoonosis
AMS	American Meteorological Society
ANCOVA	Analysis of Covariance
APHIS	Animal and Plant Health Inspection Service
CEA	Critical Equilibrium Activity
D0	Drought Stress Category 0, Abnormally Dry
D1	Drought Stress Category D1, Moderate Drought
D2	Drought Stress Category D2, Severe Drought
D3	Drought Stress Category D3, Extreme Drought
D4	Drought Stress Category D4, Exceptional Drought
GE2	Drought Stress Greater than or Equal to D2, includes D2, D3 and D4
GE3	Drought Stress Greater than or Equal to D3, includes D3 and D4
NCDC	National Climatic Data Center
NDMC	National Drought Mitigation Center
NFDRS	National Fire Danger Rating System
NOAA	National Oceanic and Atmospheric Administration
PDHI	Palmer Hydrological Drought Index
PDSI	Palmer Drought Severity Index
PROC	Procedure (SAS)

PROC MIXED	Procedure Mixed Model (SAS)
RH	Relative Humidity
RMSF	Rocky Mountain Spotted Fever
SAS	Statistical Analysis Software
SD	Saturation Deficit
SFG	Spotted Fever Group
TAHC	Texas Animal Health Commission
TAME	Tick Adverse Moisture Event
USDA	United States Department of Agriculture
USDM	United States Drought Monitor
WFAS	Wildland Fire Assessment System
WT	White-Tailed Deer

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1. INTRODUCTION

1.1 Abiotic Factors Affecting Ixodid Tick Survivorship

Desiccation is the greatest risk to maximum survivorship during the off-host phase of ixodid tick (Acari: Ixodidae) life cycles (Knülle and Rudolph 1982, Needham and Teel 1991). Greater than 90% of the life histories of one-, two-, and three-host ixodid ticks is spent at the soil-vegetation interface in microclimates of vegetation communities where they have dropped from completing a blood meal from their last host. Tick development including egg deposition, incubation and eclosion, as well as molting of blood-fed larvae and nymphs are completed in these microclimates. The subsequent tick stages must await the passage of their next host to continue the developmental cycle while surviving in the habitat microenvironment (Needham and Teel 1991, Apanaskevich and Oliver Jr 2013) .

Ixodid ticks are subject to passive water loss through the cuticle and thus their large surface area-to-volume ratio increases this risk of desiccation. Ticks mitigate water loss during host questing periods on grasses and shrubs by returning to more moisture rich microenvironments (duff or leaf litter) and initiating active water uptake (Semtner et al. 1971a, Semtner et al. 1971b, Needham and Teel 1991). Active water uptake is an energy requiring physiological process through which ticks recover water-loss by absorbing moisture from subsaturated air (Needham and Teel 1991). This process involves the deposition of salts on the hypostome from specialized salivary gland cells when the water vapor activity (% Relative Humidity (RH)/100) is below the

Critical Equilibrium Activity (CEA), defined as the point at which the rate of passive water loss and gain are equal (Needham and Teel 1986). These salts deliquesce when the water vapor activity exceeds the CEA enabling ticks to imbibe liquid water (Needham and Teel 1991, Sigal et al. 1991, Yoder and Spielman 1992). Tick survivorship thus depends heavily upon the dynamics of oscillations between periods of water loss and recovery. The variation of environmental conditions among heterogeneous habitats across a landscape is recognized as one of the driving components of both temporal and spatial tick distribution (Semtner et al. 1971b, Fleetwood 1985).

Ixodid tick development, survivorship and population responses have typically been assessed across habitat types in association with microclimate and mesoclimate temperature, precipitation, relative humidity, dew point or vapor pressure deficits (Daniel and Černý 1967, Semtner et al. 1971b, Bertrand and Wilson 1996, Teel et al. 2010). Assessing ixodid tick populations at larger spatial scales tend to rely upon macroclimate temperature and precipitation variables, although with mixed results (Schauber et al. 2005, Barandika et al. 2006, Leschnik et al. 2008, Alonso Carne et al. 2015). Alonso-Carne et al. (2015) assessed the statistical relationship of rainfall and RH and saturation deficit (SD) to appraise tick habitat. They found a single day of rainfall does not correlate with the subsequent RH and SD 24 hours later; as such rainfall could not replace RH or SD and was not a sufficient replacement to evaluate physical processes of ticks at a regional scale. Precipitation was also found to not be a major influence in seasonal occurrence of canine babesiosis in Central Europe by *Dermacentor* *reticulatus* (Leschnik et al. 2008). Additionally, Schauber et al (2005) found a negative relationship between total summer precipitation, lagged by 1 year, and Lyme disease incidence. However, Barandika et al. (2006) concluded that an interaction between precipitation 7-days prior to tick sampling by drag-cloth collection and the ambient temperature at the time of collection, were positively associated with collection rates for *Ixodes ricinus*, in the Basque region of Spain. Collectively, these assessments were designed to estimate population growth and expansion over relatively short periods of time and small spatial scales.

1.2 Use of Environmental Variables in Tick Modeling

The use of climate variables alone in modeling tick populations and distribution has been scrutinized. Estrada-Pena (2008) has proposed that it is possible to assess the climate in a geographic region and compare it to the ideal conditions for tick survival to deduce an "index of suitability" for the region in question. He calls this a "climate niche" and also points out shortcomings of understanding and applying this concept, his scrutiny includes: 1) macroclimate may not account for key microclimate variables necessary for survival in the ecological community, 2) tick "niche" data assume homogeneous tick distribution within the habitat, 3) large region models may have less local predictability and 4) regional environmental variation may cause response variation in the tick (Estrada-Peña 2008). Likewise, Schauber et al (2005) found that weather variables with a one year lag, mean summer and winter temperature and mean total summer precipitation, showed only a weak causal relationship with Lyme disease incidence, while the Palmer Hydrologic Drought Index showed significant positive predictive value based on a two year lag analysis, but only in three out of eight states studied (Schauber et al. 2005). These results, coupled with additional biotic factor analyses, led them to conclude that biotic and abiotic factors both contribute to the incidence of Lyme disease and must be included together for reliable risk modeling predictions.

Most recently, Berger et al (2014) provided the largest temporal and spatial scale assessment of tick abundance based upon a single abiotic factor. They investigated the relationship of time below 82% RH, an equivalent estimate of the CEA for *Ixodes scapularis* nymphs (Rodgers et al. 2007), and the estimated abundance of nymphs in the statewide survey over the same period in Rhode Island. They transformed 14-year macroclimate RH data sets from three airport weather stations using a microclimate correction established by monitoring RH in replicated leaf litter habitats, then calculated a physiological stress factor called Tick Adverse Moisture Events or TAMEs for the month of June, coinciding with the first month of nymph activity. Their observation that the trend of low annual nymph collections being associated with an increased number of TAMEs, was substantiated by results of linear regression (coefficient=-69.57, SE-27.66, $P<0.027$). The results were not significantly improved when additional parameters for degree of winter severity, TAMEs for June in prior years were added to the models analyzed.

Ticks are extremely susceptible to abiotic conditions, and desiccation can have a profound impact on their survival, thus a review of drought concepts and currently available drought indices is appropriate.

1.3 Current Drought Indices and Indicators

There are four categories of drought as described by The American Meteorological Society: 1) meteorological or climatological 2) agricultural, 3) hydrological and 4) socio-economic drought (AMS 2013). Meteorological drought is the absence or reduction of precipitation over a prolonged period of time, while agricultural drought takes into account the needs of plants versus the availability of water in the soil for successful growth. Hydrological drought involves surface and sub-surface water effects such as river flow or snowmelt. Socio-economic drought associates the impact of the other three categories to human activities, such as how the lack of crops affects the economy or how low river flow decreases summer revenue from water activities (Heim Jr 2002, Wilhite and Buchanan-Smith 2005, Mishra and Singh 2010). The environmental impact on vector-borne diseases can also be included in socio-economic drought impacts, such that if drought decreases vector populations, a favorable economic outcome from an animal husbandry or disease standpoint could result. My focus is on meteorological drought and its socio-economic effects with respect to ixodid ticks. The following is a brief overview of two major drought indices and a third methodology for assessing drought conditions, as it relates to the risk of forest fires.

1.3.1 Palmers Drought Severity Index (PDSI)

"The Palmer Drought Severity Index (PDSI) (known operationally as the Palmer Drought Index (PDI)" (NCDC:NOAA 2014a) and the U.S. Drought Monitor (USDM) are two major drought indices developed to provide a drought characterization across space and time (Heim Jr 2002). The PDSI was developed in 1965 and is used to measure the intensity and duration of long-term drought. It relies on precipitation and temperature from previous time periods, not just precipitation over a fixed interval (NCDC:NOAA 2013). This index takes into account precipitation, evapotranspiration and soil moisture content (Palmer 1965). "The "Palmer Index" refers collectively to three indices: PDSI, Palmer Hydrological Drought Index (PDHI) and the Palmer Z Index" (Heim Jr 2002). The index values are related to drought severity as follows: -4.00 and below = Extreme drought, -3.00 to -3.99 = Severe drought, -2.00 to -2.00 = Moderate drought, -1.99 to +1.99 = Mid-range, and +2.00 and above = Excess moisture (NCDC:NOAA 2013). The PDSI is effective in prompting the beginning or end of drought response actions or for measuring the effects of adverse soil moisture conditions (Heim Jr 2002).

1.3.2 U.S. Drought Monitor (USDM)

The USDM was developed in 1999 and incorporates several key indices or parameters and auxiliary drought indicators for a more robust drought monitoring system (Svoboda 2000). The USDM dryness levels are given as five categories, D0 to D4, labeled as Abnormally Dry, Moderate Drought, Severe Drought, Extreme Drought and

Exceptional Drought, respectfully. The five key indicators are: 1) Palmer Drought Index, 2) Climate Prediction Center (CPC) Soil Moisture Model (Percentiles), 3) United States Geological Survey (USGS) Weekly Streamflow (Percentiles), 4) Standardized Precipitation Index (SPI) and 5) Objective Short and Long-term Drought Indicator Blends (Percentiles). While an identical drought severity level will not necessarily be shown by all five indicators, the USDM drought category is indicative of the majority of the indicators, combined with local and expert input (NDMC 2014). The USDM also utilizes auxiliary indicators, mainly during the growing season, that include: the United States Department of Agriculture (USDA), National Agricultural Statistics Services (NASS) Topsoil Moisture, Keetch-Byram Drought Index (KBDI), and National Oceanic Atmospheric Administration (NOAA), National Environmental Satellite, Data, Information Service (NESDIS) satellite Vegetation Health Indices (NDMC 2014). Despite similarities between the PDI and USDM, the added indicators used for the USDM allow for better refinement and discernment of drought conditions across geographical regions.

1.3.3 National Fire Danger Rating System (NFDRS) – Dead Fuel Moisture

A third method to measure drought is the National Fire Danger Rating System (NFDRS) and is used by the United States Forest Service - Wildland Fire Assessment System (WFAS). Development of the NFDRS began in 1968, and it became operational in 1972 (Cohen and Deeming 1985). NFDRS is related to the moisture in dead fuels, which are dead, woody vegetation components in the environment such as leaves, twigs, branches, and trees, and is expressed as a percentage of dry weight of that specific fuel (NCDC:NOAA 2014b). Dead fuel moisture is reactive to environmental conditions including weather and vegetation type. Therefore, when assessing the lag time or class of dead fuel moisture, NFDRS also considers: temperature, humidity, cloudiness, day length and hours of rain (Burgan et al. 1997, USFS-WFAS 2014). "The dead fuel moisture threshold (10–hour, 100–hour, or 1,000–hour), called a time lag, is based upon how long it would take for 2/3 of the dead fuel to respond to atmospheric moisture" (NCDC:NOAA 2014b). Once vegetation contains 30% or less of its original moisture content it is considered "dead fuel" rather than "live fuel" and it is used to assess the likelihood of a forest fire given past and present environmental conditions. Dead fuels are broken into four classes based on the diameter of the vegetation in question; three of the classes are show below in Table 1. A fourth class is 1-hr lag time for vegetation of less than 0.25 inches (6.4 mm) in diameter and it is computed from observation time, humidity and cloudiness. Small twigs, grasses and leaf litter would be placed in this class of dead fuel.

Table 1. Definition of the dead fuel moisture time lag classes. (NCDC:NOAA 2014b)

TIME LAG	FUEL SIZE	DETERMINATION
10–hour	0.25 to 1 inch diameter	Computed from observation time temperature, humidity, and cloudiness. Can also be an observed value, from a standard set of fuel sticks that are weighed as part of the fire weather observation.
100–hour	1 to 3 inches diameter	Computed from 24–hour average conditions composed of day length, hours of rain, and daily temperature/humidity ranges.
1000–hour	3 to 8 inches diameter	Computed from a 7–day average conditions composed of day length, hours of rain, and daily temperature/humidity ranges.

All three indices or methods share many of the same data inputs but each is calculated or presented in a slightly different manner. Mishra and Singh (2010) compared the benefits and limitations of the PDSI and the USDM, and the USDM Classification Scheme appears to provide the broadest integration of drought parameters and indicators, within an appropriate time scale, to best assess the impact of environmental conditions on the life history of A. maculatum.

1.4 *Amblyomma maculatum* – A Tick of Concern

The Gulf Coast tick, *Amblyomma maculatum* Koch (Acari: Ixodidae), is a univoltine, three-host ixodid tick with egg, larval, nymphal and adult life stages. The seasonal phenology of *A. maculatum* in Texas involves three overlapping cycles of molting, questing, feeding and/or oviposition, one for each life stage: 1) Adults are active between June and November, with their peak is during August and September, 2) Larvae are active from November to January, and finally 3) Nymphs are active between January and June (Teel et al. 2010). Overall *A. maculatum* shows a wide array of host preferences including birds, lagomorphs, rodents, carnivores, ungulates and humans (Teel et al. 2010). The geographical range of *Amblyomma maculatum* in the U.S. is predominantly along the Gulf Coast region from Texas to Florida, and it has also been documented on the Eastern seaboard from South Carolina to Virginia and in inland areas including Kentucky, Tennessee, Arkansas, Oklahoma and Kansas (Teel et al. 2010, Paddock and Goddard 2015). This tick species is originally described as being distributed within 100-150 miles (160 - 241 km) (Hooker et al. 1912) of the coast, and more inland occurrences are attributed to cattle or bird movement and/or patterns of drought and precipitation (Teel et al. 2010, Paddock and Goddard 2015).

Amblyomma maculatum spend an aggregate of approximately 23 days feeding on separate hosts as larvae, nymphs and adults, while the remainder, more than 90% of their life expectancy, is spent off-host near the soil-vegetation interface. In this environment they are susceptible to abiotic factors making them susceptible to desiccation. They also demonstrate neither the propensity nor the ability to "drink" water, so they are highly reliant on environmental conditions to facilitate active water vapor uptake (Hair et al. 1975, Needham and Teel 1991, Yoder et al. 2008). Yoder et al (2008) identified the larval stage of *A. maculatum* as the stadium most susceptible to desiccation, although nymphs appear as susceptible based on laboratory and field experiments as outlined by Teel et al (2010). Extreme adverse environmental conditions, such as drought could potentially disrupt the immature life stages of *A. maculatum*.

1.4.1 Factor in Primary Screw Worm Infestation in Texas

Amblyomma maculatum was implicated as a contributing factor to the success of primary screwworm fly, *Cochliomyia hominivorax* Coquerel (Diptera: Calliphoridae), infestation in cattle in the Gulf Coast states including Texas (Graham and Hourrigan 1977). Gulf Coast ticks prefer the head and ears of cattle, and their feeding caused significant damage including inflammation, open wounds and abscesses that female screwworm flies were drawn to for oviposition sites. The larvae subsequently caused further damage resulting in ear drooping and deformations known as “gotch ear” that were permanent and affected both the cow physically and its sale barn revenue. Screwworm infestations resulted in significant animal losses and veterinary care through the first part of the 20th century (Bishop and Hixson 1936, Dove and Bishopp 1936, Spicer and Dove 1938). The role of Gulf Coast ticks in predisposing animals to screwworm infestation encouraged research and development of control measures for *A. maculatum* such as insecticidal ear tags, bands and ear smears (Spicer and Dove 1938, Gladney 1976, Gladney et al. 1977). These methods, while providing short-term relief and control, were expensive both monetarily and with respect to manpower. Ultimately, mass rearing and release of sterile male screwworm flies to mate with wild females led to the eradication of screwworms in Texas by the late 1970’s (Graham and Hourrigan 1977). In addition to the economic impact of screwworm infestations and *A. maculatum*’s role, researchers looked at other physical effects including changes in weight gain and blood composition in both pasture and feedlot conditions. The results indicated infested cattle had reduced weight gain and changes in blood composition, by significant amounts, as compared to control animals, in addition to the physical deformation to the ears (Williams et al. 1977, Stacey et al. 1978, Williams et al. 1978, Riley et al. 1995). Reduced weight gain and physical deformities translate to lower selling price per pound at the feedlot for ranchers, imposing another economic burden due to tick infestation. *Amblyomma maculatum* continues to be an economic concern to the cattle industry today but it also serves as an important medical concern.

1.4.2 Human Impact – Vector of Rickettsia parkeri

The Gulf Coast tick was first identified as an arthropod of medical concern after an unknown rickettsia-like infectious agent was isolated from a specimen collected in Liberty County, Texas in 1937 (Parker et al. 1939). Additional work later confirmed the agent to be part of the Spotted Fever Group (SFG) rickettsia, similar to Rocky Mountain Spotted Fever (RMSF) albeit a much milder form, and was classified as *Rickettsia parkeri* (Lackman et al. 1949, Lackman et al. 1965). Clinical features of *R. parkeri* can include, but are not limited to: fever, eschar, rash including erythematous papules and tender or ulcerated pustules, myalgia, arthralgia, headache and lymphadenopathy (Paddock et al. 2004, Paddock et al. 2008). The first laboratory confirmed case of *R. parkeri* in humans did not occur until 2002, in a man from the coastal area of Tidewater, Virginia (Paddock et al. 2004) and there have been at least 20 cases documented since (Paddock et al. 2004, Paddock et al. 2008, Cragun et al. 2010, Paddock et al. 2010). It is theorized, based on clinical manifestation and epidemiologic findings, that many past diagnoses of RMSF could in fact be attributed to *R. parkeri* mainly due to the nonspecific testing protocols available at the time (Cragun et al. 2010, Paddock et al. 2010). Additionally, *R. parkeri* has been isolated from ticks in Georgia, Florida, Kentucky, Mississippi, Oklahoma, South Carolina, Texas, and Virginia, indicating *R. parkeri* potentially exists anywhere *A. maculatum* is found (Sumner et al. 2007, Whitman et al. 2007, Goddard and Varela-Stokes 2009, Paddock et al. 2010).

1.4.3 Animal Impact – Vector of Hepatazoon americanum

Amblyomma maculatum also vectors animal pathogens that are of concern and/or have possible economic impacts. *Amblyomma maculatum* has been identified as the vector for an emerging disease in dogs known as American canine hepatozoonosis (ACH), caused by *Hepatozoon americanum*, a highly debilitating *Hepatozoon* species in the phylum Apicomplexa, parasitic protozoans (Ewing and Panciera 2003, Johnson et al. 2009). Another *Hepatozoon* species, *Hepatozoon canis*, vectored by *Rhipicephalus sanguineus* Latreille (Acari: Ixodidae), was previously reported in several countries in the Eastern Hemisphere as early as 1905; however, the first natural case of hepatozoonosis in canines in the Western Hemisphere was found in a coyote in Texas in 1977 (Davis et al. 1978). At that time, it was identified only as a *Hepatozoon* species due to its novelty in the Western Hemisphere and tentative presumption that it was the first appearance of H. canis in the U.S. In 1997 Auburn researchers identified *H. americanum* as a new *Hepatozoon* species based on its unique characteristics including: clinical presentation, tissue stages and tick vector (Vincent-Johnson et al. 1997). Further, in 2000 genetic and antigenic evidence supported the separation of

H. canis and *H. americanum* at the species level (Baneth et al. 2000). The natural cycle of ACH has not been fully elucidated due to the broad host range of *A. maculatum* life stages, more specifically the larval stage. However, research shows canines can acquire ACH primarily through ingestion of *A. maculatum* both through self-grooming and ingestion of adults or nymphs and ingestion of prey infested with infected ticks (Ewing and Panciera 2003, Johnson et al. 2009). Infected dogs exhibit symptoms including: fever, stiffness, depression, lethargy, gait abnormalities, muscle wasting, especially atrophy of head muscles, mucopurulent ocular discharge and death, if untreated (Ewing and Panciera 2003, Baneth 2011). The impact on companion animals and their owners is cause for concern; however, *H. americanum* has not to date been identified as a zoonotic issue. Nonetheless, *A. maculatum* is relevant regarding a pathogen that is potentially economically damaging to our cattle industry if introduced to the U.S.

1.4.4 Veterinary and Economic Impact – Vector Potential for Ehrlichia ruminantium

Amblyomma maculatum has demonstrated highly effective vector potential for *Ehrlichia ruminantium* (Mahan et al. 2000). *Ehrlichia ruminantium*, previously classified as *Rickettsia ruminantium* and *Cowdria ruminantium*, is commonly known as Heartwater and is transmitted by ticks of the genus *Amblyomma* (Allsopp 2010). It is a gram-negative, obligatory intracellular proteobacterium (Rickettsiales: Anaplasmatacae) (Kasari et al. 2010). Onset of heartwater, in the acute form, includes a transient but persistent febrile state accompanied by listlessness, lack of appetite, a moist cough, and cyanotic membranes with dyspnea accompanied by central nervous system symptoms, and is typically fatal in less than a week of onset of clinical signs (Kasari et al. 2010). This disease is endemic to sub-Saharan Africa and was first described in 1838, although it was not recognized to be a tick-borne disease until 1900 with the causative agent identified in 1925 (Allsopp 2010, Kasari et al. 2010). It has also been described in the Caribbean, including the French Antillean islands of Guadeloupe, Marie Galante and Antigua. Additionally, *A. variegatum* Fabricius (Acari:Ixodidae), a primary vector for *E. ruminantium,* has become established on 14 -18 additional islands including Martinique, St. Kitts, St. Martin St. Lucia, Nevis and Vieques (Garris 1984, Barre and Garris 1990, Allsopp 2010, Kasari et al. 2010, Beati et al. 2012). The expansion of *A. variegatum* in the Caribbean appears to coincide with the establishment of cattle egrets, *Bubulcus ibis* Linnaeus (Ciconiiformes:Ardeidae), to the area around 1950 and is the crux for potential introduction to mainland U.S. based on migratory pathways of the cattle egret, Figure 2. (Corn et al. 1993, Kasari et al. 2010).

During laboratory experiments, *A. maculatum* nymphs obtained infection from all five Merino-Dorper cross sheep, *Ovis aries* L. (Artiodactyla:Bovidae) infected with *C. ruminantium*. Adult Gulf Coast ticks also successfully transmitted three strains of *C. ruminantium* to naïve sheep (Mahan et al. 2000). Hosts of *E. ruminantium* include a long list of wild and domestic ruminants, with the wild species being the most likely original reservoir (Allsopp 2010). There are 15 ruminant spp. in the U.S. that are suspected to be susceptible to heartwater, this includes white-tailed deer (WT), *Odocoileus virginianus* Zimmermann (Artiodactyla:Cervidae) and axis deer, *Axis axis*

Erxleben (Cetartiodactyla:Cervidae) as well as wildlife imported from Africa (Yabsley et al. 2008, Kasari et al. 2010). The migratory pathways of the cattle egret shown in

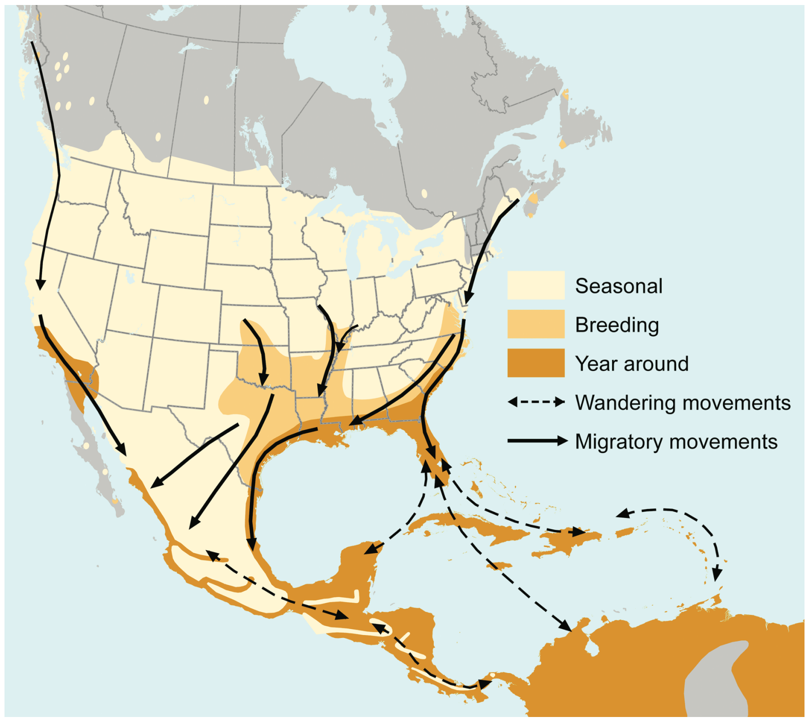

Figure 1. Migratory pathways of cattle egrets. (Reprinted with permission from Kasari et al. 2010)

Figure 2 illustrate the potential dispersal routes of *A. variegatum* into the U.S., to include Texas, and Central and South America. Cattle egrets keep in close proximity to cattle or other ruminant stock as part of their feeding and resting cycle (Kasari et al. 2010). 2010b). It would require only a small number of *A. variegatum* to introduce *E. ruminantium* into an area populated with naïve wild or domestic ruminants and *A. maculatum* could potentially replace *A. variegatum* as the vector for pathogen transmission in the U.S.

1.5 Description of Study Area

The selected study area is bounded in the East by the Texas border with Louisiana and Arkansas, and in the West roughly approximated by a line from Wichita Falls, Texas, south to the Gulf of Mexico and not inclusive of counties bordering Mexico; as these counties are the focus of intense tick surveillance in the international boundary area with Mexico, as described in section 2.1. The overall landmass of the study area (148 counties) is 128,957.5 square miles as estimated by the U.S. Drought Mitigation Center, Lincoln, Nebraska. Figure 2 depicts the study area and shows the two zones, Coastal (blue) and Inland (green); additionally, Table 2 provides a list of the counties in each zone. The coastal zone is 59,812.4 square miles and is comprised of 66 counties within approximately 100 miles (160 km) of the Gulf Coast, the basis of the common name of this tick (Bishop and Hixson 1936, Teel et al. 2010). The Inland zone consists of the remaining 82 counties with an area of 69,145.1 square miles. The Coastal zone is thought to be advantageous to tick survival resulting from moist climatic influences of the Gulf of Mexico (Teel et al. 2010). In contrast, the Inland zone of counties tends to be drier and more subject to impacts of wet/dry cycles.

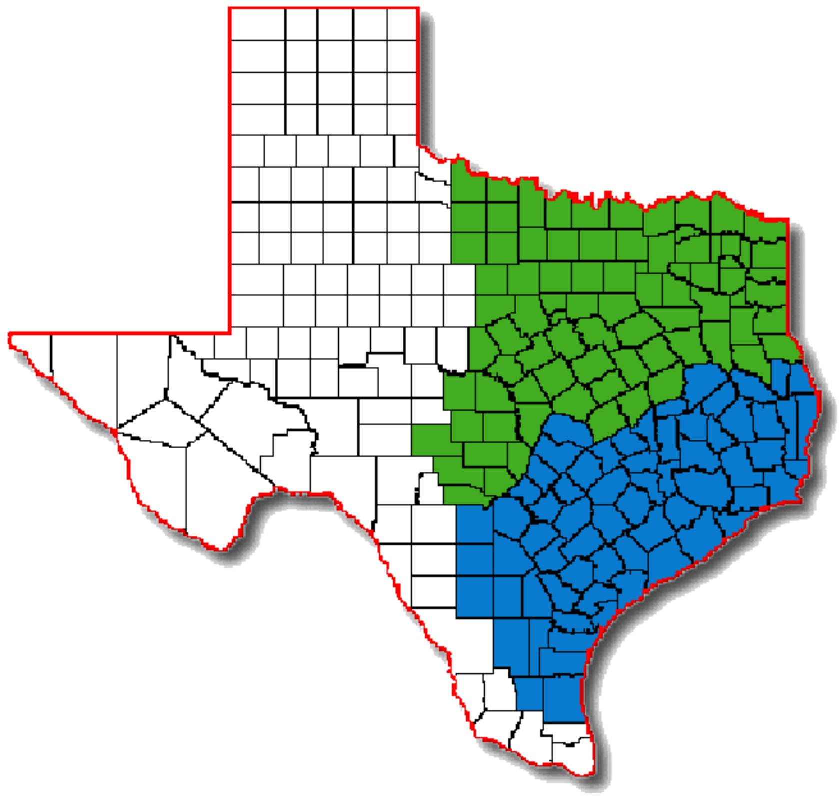

Figure 2. Texas map highlighting the study area. The Coastal zone is shown in blue and Inland zone is in green.

Table 2. Study area counties. This table provides the counties in each of the study area zones, Coastal and Inland

Study Area Counties					
Coastal	Guadalupe	Polk	Brown	Henderson	Navarro
Angelina	Hardin	Refugio	Burnet	Hill	Palo Pinto
Aransas	Harris	Sabine	Camp	Hood	Panola
Atascosa	Hays	San Augustine	Cass	Hopkins	Parker
Austin	Houston	San Jacinto	Cherokee	Hunt	Red River
Bastrop	Jackson	San Patricio	Clay	Jack	Robertson
Bee	Jasper	Travis	Collin	Johnson	Rockwall
Brazoria	Jefferson	Trinity	Comanche	Kaufman	Rusk
Brazos	Jim Wells	Tyler	Cooke	Kendall	San Saba
Brooks	Karnes	Victoria	Coryell	Kerr	Shelby
Burleson	Kenedy	Walker	Dallas	Kimble	Smith
Caldwell	Kleberg	Waller	Delta	Lamar	Somervell
Calhoun	La Salle	Washington	Denton	Lampasas	Stephens
Chambers	Lavaca	Wharton	Eastland	Leon	Tarrant
Colorado	Lee	Williamson	Ellis	Limestone	Throckmorton
Comal	Liberty	Wilson	Erath	Llano	Titus
DeWitt	Live Oak	**Inland**	Falls	Marion	Upshur
Duval	Madison	Anderson	Fannin	Mason	Van Zandt
Fayette	Matagorda	Archer	Franklin	McCulloch	Wichita
Fort Bend	McMullen	Bandera	Freestone	McLennan	Wilbarger
Frio	Medina	Baylor	Gillespie	Milam	Wise
Galveston	Montgomery	Bell	Grayson	Mills	Wood
Goliad	Newton	Blanco	Gregg	Montague	Young
Gonzales	Nueces	Bosque	Hamilton	Morris	
Grimes	Orange	Bowie	Harrison	Nacogdoches	

1.6 Study Objectives

This project is a retrospective observational study compiling and analyzing Gulf Coast tick collection records of the Texas Animal Health Commission (TAHC) and weekly national drought data in Texas for the period 2000-2014. The objectives of this study were set forth to address one main question, can Gulf Coast tick population changes in Texas be associated with categories of drought stress as defined by the U.S. Drought Monitor Classification Scheme?

The three study objectives are:

1) Compare Gulf Coast tick population changes from 2000-2014 between two adjacent geographic areas of Texas; defined as inland and coastal zones.

2) Compare drought stress from 2000-2014 between the coastal and inland Gulf Coast tick areas.

3) Test whether trends in Gulf Coast tick population changes can be explained by corresponding changes in drought stress.

2. CHARACTERIZATION OF TICK COLLECTION DATA

2.1 Introduction

The state-federal tick surveillance program provides a statewide network of animal health inspectors who are, in part, tasked with collecting tick specimens from livestock and other animals to guard against the re-introduction and establishment of the cattle fever ticks, *Rhipicephalus* (*Boophilus*) *microplus* Canestrini (Acari:Ixodidae) and *Rhipicephalus* (*Boophilus*) *annulatus* Say (Acari:Ixodidae) from Mexico (Graham and Hourrigan 1977). United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) inspectors concentrate tick surveillance activities along the international border with Mexico, while the TAHC animal health inspectors concentrate on tick surveillance activities throughout the remainder of the state. Collections of ticks are sent to the Texas State-Federal Laboratory, Austin, Texas and identified by trained, experienced technicians. For this study, only those collections containing adults or nymphs identified as *A. maculatum* are included in the analyses. Counts of collection records were used as the response variable, not the number of ticks contained in the collections. All tick collection records used in this study were from the state-federal tick surveillance program and were obtained from the TAHC for the period 2000-2014, a 15-year timeframe. A "collection" is strictly a single laboratory submission of a sample consisting of 1 or more tick specimens for identification. The "collection" will become the count statistic not the number of ticks contained in the submission. Collection records, both carbon and photocopies, were used in combination with electronic records from the TAHC to transcribe the records into electronic spreadsheets for analysis and archival purposes for future access. Each collection has a unique sequential “referral number” for the collection year and is the identifying item for that record, as such submissions could be collected in a different month than they were identified. This includes end of year collections that were not identified until the following calendar year, thus receiving a referral number associated with the new year. Tick identifications were usually annotated within two weeks of collection, however delays were noted from one to six months after the collection date in some cases. For the purposes of this project and based on tick phenology all collections were grouped and analyzed based on their collection date and county collected in, not their classification or identification date. In some cases information on the forms was missing or unreadable, in these instances the information was filled-in based on surrounding reports and other reports from the same inspector; including collection date, county collected in and species collected from. When information could not be derived from surrounding data these records were annotated and discarded from the data set.

2.2 Methods

2.2.1 Data Characterization

There were a total of 35,224 collections for all counties in the state of Texas over the 15-year study period (Appendix A-1). Of these records there were less than one percent, 12 records total, which were discarded initially due to incomplete or unreadable data (e.g. missing county name), TAHC personnel omitted the record or the record was missing from both the paper and electronic files and could not be located. A total of 10,796 (31%) contained adult *Amblyomma maculatum* for all collections in Texas. Four collections of adult *A. maculatum* also contained nymphs of this species collected in the months of November 2007, January 2008 and October 2011, all in Brooks County. Additionally, these collections were made from two cervids and one equine.

Of the 10,796 collections, 9,195 (85%) were confirmed to be *Amblyomma maculatum*, within the 148 county study area (Appendix A-4). Collections for the study area were further divided with 7,936 (86%) in the Coastal zone and 1,259 (14%) in the Inland zone (Appendix A-5). The number of tick collections by month (Appendix A-2 and A-3) shows a close mirror of *A. maculatum* phenology. On average, there are very few collections from January to March, with a steady increase starting in March, peaking in August and then steadily declining from September through December. See Appendix C for yearly tick collections graphed by month for the study area that show the same trend following the published phenology of *A. maculatum*. Finally, Appendix E provides a breakdown of the study area collections by host species. Cattle (bovine) comprised 8,901 (96.8%) of the 9,195 collections, while dogs (canine) consisted of 149 collections (1.62%) and the remaining 145 collections (1.58%) consisted of 10 other species or collection sources. The study area breakdown for the Coastal zone was as follows: a total of 7945 collections (86% of study area collections) of which 7794 (98%) were bovine, 70 (1%) were canine and the remaining 81 (1%) were from 8 other species or collection source. The Inland zone consisted of 1250 collections (14% of study area collections) of which 1107 (89%) were bovine, 79 (6%) were canine and the remaining

64 (5%) were from 4 other species or collection source. Although collections were possible for all 148 counties, 23 counties did not have any collections across the 15-year study period. As such, those 23 counties do not contribute to the analysis regarding tick collections.

2.2.2 Data Analysis – Repeated Measures Modeling Using PROC MIXED

The data for tick collections by year and location were subjected to analysis by using a repeated measures model procedure, PROC MIXED, of Statistical Analysis Software (SAS) (SAS Institute Inc. 2011), model: Ticks = Year + Location + Year*Location, where ticks is the number of tick collections in a given county in a given year. This model was used to assess correlation of the number of tick collections across the years within a given county. Two analyses were conducted to test for differences in tick collection by year, by location of coastal or inland and for an interaction between year and location with the county of collection serving as the replication source. The first analysis was for tick collections for the entire calendar year while the second analysis focused on collections from June to November, as related to the period of peak adult tick activity

2.3 Results

The PROC MIXED results for the model (Ticks = Year + Location + Year*Location) indicate there is an interaction between Year and Location for the 12-month period, but not for the June – November period. This means the yearly trends for collections in Coastal and Inland zones are different (F Value = 1.82, DF 14, 694, P = 0.0328) for the 12-month period. An interaction plot of the Least Square (LS) Means illustrates their different patterns, Appendix B-1. However, the analysis for collections from June to November indicates no interaction between Year and Location (P = 0.0985) and only Location is highly significant (F Value = 21.71, DF 1, 118, P = <0.0001). It should be noted that the lack of interaction for this shorter period is a difference of just 71 observations, 776 observations out of the possible 847 met the criteria during the June – November period, whereas the 12-month period contained all 847 observations. Appendix B-2 shows the interaction plot for the LS Means for the June-to-November period.

2.4 Discussion

The results indicate that the difference between coastal and inland collections containing adult Gulf Coast ticks are not staying the same across the years, they are changing. The interaction for the 12-month period shows that collections in the study area are affected by the year in which they occur and their location. The lack of interaction for the June-to-November period along with location being highly significant, however points to location being of greater importance during the peak adult period. Overall, the coastal zone consistently returns higher numbers of collections than the inland zone, which support the Gulf Coast tick's natural preference for the climate and geography within approximately 100-150 miles (160-241 km) of the coast.

The comparison of host species represented among tick collections between coastal and inland locations reflects the priority focus of animal health inspectors on cattle through auction facilities. The percentage of host species type between locations was approximately equal. It is worth noting that the second most frequent host type in both coastal and inland zones was canine. Working canines routinely accompany people, cattle, and horses to auction facilities and may have been the source for the frequency of tick collections from this host type.

The collection and the identification of four *A. maculatum* nymphs though small in number, is of considerable significance. These are very small ticks and in the engorged state just reach what is deemed a "detectable" size of 8mm by a human physically examining a host animal (Wharton and Utech 1970, Palmer et al. 1976). These collections were made during the normal seasonal phenology for the nymphs, and were collected with adult ticks, from cervids and equids. *Amblyomma maculatum* is recognized as having vector potential for *E. ruminantium* based on laboratory transmission trials, but field evidence of nymphal feeding on cattle, or other ruminants, has been lacking. These collections are supportive evidence of vector capacity of *A. maculatum* reinforces the concern of the nymph's possible role in vectoring *E. ruminantium* if it were introduced to the U.S.

There are other conditions that could artificially influence the number of or the distribution of tick collections in the study area, although they would more than likely contribute to the increase of tick collections, not detract from them. The number of tick collections can be affected by factors such as the presence or frequency of livestock auctions, availability of inspectors and the number of cattle scratched for ticks.

Livestock markets typically hold sale auctions one time per week and several hundred cattle can move through a market on those days. Inspectors do not put their hands on every single animal going to sale and some inspectors cover more than one auction on the same day or travel great distances between consecutive inspection days, this undoubtedly leaves room for under estimation of tick collections. Good record keeping and thorough inspections also play a role in in collections. This data set contained numerous records with missing information that either had to be estimated or left blank causing the record to be discarded. Despite these influencing factors, the TAHC inspection program provides the best and currently, the only means possible by which to glean an understanding of the population dynamics of *A. maculatum* in this region of Texas.

3. CHARACTERIZATION OF U.S. DROUGHT MONITOR DATA

3.1 Introduction

The USDM has proven to be a useful and powerful entity since its inception 16 years ago by utilizing the five key indicators previously described in combination with numerous auxiliary indicators, used primarily during growing season. Texas is fortunate to have crop production nearly year round, resulting in a steady flow of auxiliary and local data input for the USDM system for this region. The USDM compiles the drought data and releases it on a weekly basis, with a cutoff of 8:00 am EDT, Tuesday and a release date of the map and statistics by 8:30 am EDT, Thursday of the same week (NDMC 2014). The data can be accessed by the general public online and provides the most current drought overview at a national, regional, and state level and it also includes major U.S. watersheds as well. The choice to use USDM data is largely based on the fact that it takes into consideration several drought indices or monitoring tools that we consider very compatible with assessing the environmental conditions that would impact the population dynamics of the Gulf Coast tick. These include their use of KBDI, vegetation indices, SPI and PDI, just to name a few. They also include short and long-term drought indicators in their model that provides added richness when considering other environmental factors that can affect the success of a tick generation. The Drought Mitigation Center in Lincoln, Nebraska generously provided us with the weekly drought data for the state of Texas from January 2000 through December 2014 for our project.

3.2 Methods

3.2.1 Data Characterization

The data were received in comma-separated value (.csv) format, arranged categorically for each county by drought stress categories, None - D4 and provided county landmass in square miles, the percent of the county land area in each drought stress category and total county area in each drought stress category, see example in Table 3.

Table 3. U.S. Drought Monitor data. This example shows the USDM data in categorical format by county, with county area and area and percent of area by drought stress level. The data below is from release date 7/1/08.

County	County Area	None (%)	None Area	D0 (%)	D0 Area	D1 (%)	D1 Area	D2 (%)	D2 Area	D3 (%)	D3 Area	D4 (%)	D4 Area
Hays	673.9	0	0	0	0	0	0	0.81	5.5	40.3	271.6	58.89	396.8
Hill	983.4	0	0	2.5	24.6	91.08	895.7	6.41	63.1	0	0	0	0

For each county, the sum of the drought stress categories, Nothing – D4 equal 100% and the corresponding areas sum to equal the county area. This format required no additional manipulation prior to analysis. In addition, the annual mean percentage area (in square miles) for all drought stress categories by month was also calculated, and used to create boxplots, for the inland and coastal zones to illustrate and compare the monthly mean, median and quartiles, Appendix F. The box represents the 75% - 25% area of land mass experiencing the drought stress, while the tails annotate the minimum and maximum range, the diamond in the box is the median and the horizontal black bar is the mean. Interaction plots for the LS Means of the Mean Percent Area for each drought stress category or combination can be found in Appendix G.

3.2.2 Data Analysis – Repeated Measures Modeling Using PROC MIXED

Analyses were performed on all drought stress categories, D0, D1, D2, D3, D4 and combinations of D2 and greater (GE2), and D3 and greater (GE3) to characterize the USDM data across the study area and between the inland and coastal zones. The inland and coastal zones differ by a little less than ten thousand square miles in land area, 69,145.1 and 59,812.4, respectfully. To account for the difference in land area, the yearly mean percentage area (the repeated measurement) for each drought stress category, by county (replication source), was calculated based on the inland or coastal zone area not the county area. The repeated measures model procedure, PROC MIXED, of SAS (SAS Institute Inc. 2011) was utilized, the model being: Mean % Area of Drought Stress Category (X) = Year + Location + Year*Location. This model was used to account for the correlation of the amount of drought stress across the years within a given county.

3.3 Results

The results showed highly significant interaction between Year and Location across all seven drought stress categories or combinations examined, see Table 4 below for values. The interaction plots in Appendix G depict the changing percentage area for drought categories between the Coastal and Inland zones across the years. For example,

Appendix G-6 for GE2, shows the Inland zone had greater drought stress from 2004 – 2007 and 2013 - 2014 than the Coastal zone, while the Costal zone had more from 2007 – 2012.

Table 4. Interaction results for all drought stress categories

Drought Stress Category	Effect	Degrees of Freedom		F Value	P Value
D0	Year*Location	14	2044	20.63	<0.0001
D1	Year*Location	14	2044	13.17	<0.0001
D2	Year*Location	14	2044	20.1	<0.0001
D3	Year*Location	14	2044	23.24	<0.0001
D4	Year*Location	14	2044	51.29	<0.0001
GE2	Year*Location	14	2044	24.72	<0.0001
GE3	Year*Location	14	2044	29.45	<0.0001

3.4 Discussion

The repeated measures analysis did not provide much insight into the differences of the drought stress categories across the Coastal and Inland zones, although it did validate that the mean percent area in drought stress categories is impacted by both location and the year experiencing the drought. The interaction plot trends show that the mean percent area of drought stress GE2 is not consistent across the 15 years for the Coastal and Inland zone. These trends can be seen across the majority of the seven drought stress categories, with D4 being the one exception where the Coastal D4 drought stress was consistently higher than the Inland zone drought stress across the 15-year study period. That may sound ominous, but a glance at the D4 boxplot, Appendix F-5, shows the mean percentage area of D4 drought stress accounted for a fairly low amount of area across the study period, collectively. Overall, the analysis showed that the mean percent area of drought stress in the study area changes frequently, the interaction plots, although the cyclic nature of drought can be more easily visualized using the boxplots, such as D0 and GE2.

In addition to the data analysis results showing the dynamic nature of drought, a quick glance at a years worth of USDM maps across the same month easily shows the fluctuating nature of drought. Two plates of USDM maps are in Appendix H and they depict February and September across the 15-year study period. While West Texas often has considerable drought, it is worth noting the drought stress conditions in Central to East Texas, encompassing the study area. It is interesting to see in some years how little drought stress there was in February 2011 but by September 2011 the entire map has changed. These two plates provide a good reference for the ever-changing drought stress across the years, sometimes being in the middle of it hides the overall dynamics.

4. COLLECTIVE ANALYSIS OF TICK COLLECTION AND USDM DATA

4.1 Introduction

The hypothesis of Objective 3, that year-to-year trends in population changes in the Gulf Coast tick can be explained by changes in drought stress was tested by comparing tick collections from selected livestock auctions with associated drought stress in the study area. Livestock auctions serve producers in the county of the auction location as well as the surrounding counties. Tick collection data associated with a specific livestock auction location is considered to be an estimate of the Gulf Coast tick population change over time of the central and surrounding counties, assuming reasonable and sustained collection effort. As such, TAHC personnel were consulted and requested to provide a list of counties with auctions in each zone that met two main criteria: 1) they had auctions that primarily sell cattle and 2) auctions that had reliable, diligent inspection for the period of 1990 – 2014, as defined by: consistent coverage by inspectors and consistent submission of tick collections. After these criteria were satisfied, a total of 29 inland and 17 coastal counties were identified and designated "primary" counties for the analysis. The drought stress category GE2 was selected based on unpublished preliminary data suggesting it had a greater impact than the other drought stress categories.

4.2 Methods

4.2.1 County Clusters

County clusters were created as the replication source within the Coastal and Inland zones. It was necessary to construct a means by which to best relate the geographical area from which cattle are most likely marketed to the "primary" counties (principal auctions) and the corresponding extent of drought stress affecting ticks during the off-host phase of the life cycle. TAHC personnel shared, that based on their experience, most cattle (>90%) are brought to an auction either in their originating county or one just adjacent. This is largely based on the cost to haul cattle to auction, the price received at auction and which day the auction is held, weekday versus a weekend. Based on this assumption, the primary counties were used as the center of a county "cluster". Each county "cluster" consisted of a "primary" county and all adjacent counties sharing its border and the clusters became the replication source within the Coastal (17 clusters) and the Inland (29 clusters) zone. A given county may be in more than one cluster such that: primary counties can also be considered as adjacent counties, inland and coastal counties can be in clusters together, mainly where they occur along the north-south dissecting boundary, and not all counties in the study area will be in a cluster, see Appendix I for cluster designations. Tick collections are only considered from the "primary" or center of the cluster in order to avoid double counting across multiple clusters. The drought condition however is summarized in terms of the cluster of adjacent counties to the primary county, since ticks could come from any of the adjacent counties. It was calculated by taking the weighted sum of the area under each drought stress level for each secondary county, where the weight is the percentage of each county out of the total area of the cluster. The weighted sum was used to account for the different amount of land area per cluster.

4.2.2 Data Analysis – Repeated Measures Modeling Using PROC MIXED

Three analyses were conducted to assess measures of drought that would potentially affected collections of adult *A. maculatum*. Drought monitor data for GE2 were lagged for periods based on the phenology of *A. maculatum* and associated with the 12-month tick collection period, January – December (e.g. January 2002 – December 2002) and the 6-month collection period, June – November (e.g. June 2002 – November 2002). The lagged drought stress periods were: 1) a 6-month period beginning in October of the first year and continuing until March of the following year (e.g. October 2001 – March 2002) to assess the impact during the prior immature period, 2) a one year lag (e.g. October 2001 – September 2002 for impact on the tick collection periods identified above), and 3) a two year lag (e.g. October 2000 – September 2002 for impact on the same collection periods), Appendix J -1 provides a visual explanation of the above example. The 1-year and 2-year time lags encompass one and two-generation times for *A. maculatum*, respectfully. Complete drought stress lag data analysis could not be performed for tick collections prior to October 2002 due to limitation of available USDM data. The Drought Index by county cluster was used as a covariate to model changes in Gulf Coast tick populations over time using a repeated measures model procedure, PROC MIXED, of SAS (SAS Institute Inc. 2011). The model is Gulf Coast

Tick Collection (# of adult tick collections) = Treatment Zone (Coastal vs Inland) + Year + Cluster Weighted Sum of Area of Drought Stress (environment) + Error. The county cluster data serves as the replication source. A log transformation was required for both the tick collection and drought stress data to reduce their skewedness.

A second methodology relating to classification of the drought stress was performed; it involved converting the continuous variable (e.g. logGE2_MA2, the drought stress index) to a categorical variable (e.g. logGE2_Factor, proportion of acreage) by dividing the continuous variable into three equal levels, Low, Medium, and High. These three factors (e.g. logGE2_Factor) represent the amount of area in GE2 drought stress across the years; Appendix J – 2 is an example from the data output. All three drought stress periods examined, October – March, October – September 1-year lag and October – September 2-year lag all had varying amounts of GE2 drought stress and each required a slightly different scale to indicate low, medium and high proportions of GE2 drought stress, see Table 5

Table 5. Factor levels for logGE2 drought stress. The levels are: Oct – Mar, 1-Year Lag and 2-year lag periods. These levels represent the amount of area, square miles, in GE2 drought stress for the Coastal and Inland zone.

GE2 Oct - Mar		GE2 Oct - Sep, 1 Yr Lag		GE2 Oct - Sep, 2 Yr Lag	
Low	0.0 ~ 0.1	Low	0.0 ~ 1.0	Low	0.0 ~ 1.5
Medium	0.1 ~ 2.2	Medium	1.0 ~ 3.0	Medium	1.5 ~ 3.0
High	> 2.2	High	3.0 ~ 5.0	High	> 3.0

4.3 Results

The results from repeated measure analyses indicate that location is highly significant for both tick collection periods and all drought stress lag periods and factors, Table 6. There is also neither significant interaction between location and logGE2 (the yearly weighted area of GE2 drought stress), nor is logGE2 alone significant, with one exception. The results for the 2-year lag for GE2 drought stress (logGE2_2YL), for both the January – December and the June – November period, are moderately significant, see Table 7.

Table 6. Repeated measures analysis results for GE2 drought stress. The GE2 drought stress level lags are: Oct – Mar and 1-year lag and GE2 drought stress factor lags are: Oct – Mar, 1-year lag and 2-year lag periods.

Dependent Variable	Drought Stress	Drought Stress Period	Effect	Degrees of Freedom		F Value	P Value
Ticks (Jan-Dec)	GE2	Oct - Mar	Location	1	44	53.16	<0.0001
Ticks (Jun-Nov)						67.06	<0.0001
Ticks (Jan-Dec)	GE2	Oct - Sep (1 year lag)	Location	1	44	57.78	<0.0001
Ticks (Jun-Nov)						69.47	<0.0001

Dependent Variable	Drought Stress	Drought Stress Period	Effect	Degrees of Freedom		F Value	P Value
Ticks (Jan-Dec)	GE2 Factor	Oct - Mar	Location	1	44	67.73	<0.0001
Ticks (Jun-Nov)						82.02	<0.0001
Ticks (Jan-Dec)	GE2 Factor	Oct - Sep (1 year lag)	Location	1	44	79.63	<0.0001
Ticks (Jun-Nov)						92.96	<0.0001
Ticks (Jan-Dec)	GE2 Factor	Oct - Sep (2 year lag)	Location	1	44	79.49	<0.0001
Ticks (Jun-Nov)						93.19	<0.0001

Table 7. Repeated measures analysis results for GE2 drought stress, 2-year lag period.

Dependent Variable	Drought Stress	Drought Stress Period	Effect	Degrees of Freedom		F Value	P Value
Ticks (Jan-Dec)	GE2	Oct - Sep	logGE2-2YL	1	642	8.93	0.0029
		(2 year lag)	Location	1	44	41.78	<0.0001
Ticks (Jun-Nov)	GE2	Oct - Sep	logGE2-2YL	1	642	8.20	0.0043
		(2 year lag)	Location	1	44	54.14	<0.0001

Additionally, slope-intercept form plots illustrating *A. maculatum* collections, for both the January – December and June – November period, as related to the amount of GE2 drought stress during the October – March period, can be found in Appendix K and the repeated measures analysis results for those plots are in Table 8 below.

Table 8. Repeated measures analysis slope-intercept form results for GE2 drought stress. The GE2 drought stress level lags are Oct – Mar, 1-year lag and 2-year lag periods

GE2 Oct - Mar			GE2 Oct - Sep, 1 Yr Lag			GE2 Oct - Sep, 2 Yr Lag		
Ticks Collections, Jan - Dec			Ticks Collections, Jan - Dec			Ticks Collections, Jan - Dec		
x	Inland	Coastal	x	Inland	Coastal	x	Inland	Coastal
3	0.47331	-0.53117	3	0.56133	-0.75132	3	0.49151	-0.68493
2	0.4985	-0.5748	2	0.57192	-0.78258	2	0.58724	-0.7366
1	0.52377	-0.61839	1	0.58251	-0.81384	1	0.68297	-0.78831
0	0.54900	-0.66200	0	0.5931	-0.8451	0	0.7787	-0.84
Slope	-0.02523	0.04361	Slope	-0.01059	0.03126	Slope	-0.09573	0.05169
y-intercept	0.5490	-0.6620	y-intercept	0.5931	-0.8451	y-intercept	0.7787	-0.8400
Ticks Collections, Jun - Nov			Ticks Collections, Jun - Nov			Ticks Collections, Jun - Nov		
x	Inland	Coastal	x	Inland	Coastal	x	Inland	Coastal
3	0.40447	-0.86312	3	0.46138	-0.984167	3	0.41981	-1.23691
2	0.41748	-0.86368	2	0.4719	-1.000078	2	0.48134	-1.19774
1	0.43049	-0.86424	1	0.48246	-1.015989	1	0.54287	-1.15857
0	0.4435	-0.8648	0	0.493	-1.0319	0	0.6044	-1.1194
Slope	-0.01301	0.00056	Slope	-0.01054	0.015911	Slope	-0.06153	-0.03917
y-intercept	0.4435	-0.8648	y-intercept	0.493	-1.0319	y-intercept	0.6044	-1.1194

The LS Means plots depicting *A. maculatum* collections, for both the January – December and June – November period, as related to the amount of GE2 drought stress factors (Low, Medium and High) during the October – March period, can be found in Appendix L and the repeated measures analysis results for those plots are in Table 9 below.

Table 9. Repeated measures analysis least square means results for GE2 drought stress factors. The factors are: Low, Medium and High, and the lag periods are: Oct – Mar, 1-year lag and 2-year lag.

GE2 Oct - Mar			GE2 Oct - Sep, 1 Yr Lag			GE2 Oct - Sep, 2 Yr Lag		
Ticks Collections, Jan - Dec			Ticks Collections, Jan - Dec			Ticks Collections, Jan - Dec		
Proportion of Acreage	Coastal	Inland	Proportion of Acreage	Coastal	Inland	Proportion of Acreage	Coastal	Inland
Low	1.6576	0.52610	Low	2.0218	0.6011	Low	2.1427	0.6740
Medium	1.8977	0.5177	Medium	2.1116	0.5530	Medium	2.0609	0.5548
High	1.68560	0.50230	High	2.0046	0.5705	High	1.9874	0.4988
Ticks Collections, Jun - Nov			Ticks Collections, Jun - Nov			Ticks Collections, Jun - Nov		
Proportion of Acreage	Coastal	Inland	Proportion of Acreage	Coastal	Inland	Proportion of Acreage	Coastal	Inland
Low	1.6645	0.4381	Low	2.0096	0.5216	Low	2.1588	0.5368
Medium	1.8531	0.3960	Medium	2.0746	0.4255	Medium	1.9967	0.4481
High	1.6269	0.4385	High	1.9283	0.484	High	1.9315	0.4457

4.4 Discussion

The results provide two key issues to explore regarding the interaction between GE2 drought stress and *A. maculatum* collections, 1) the difference of 2-year lag GE2 drought stress as compared to either other period and 2) possible sources of noise in the data. The GE2 drought stress was only significant at the 2-year lag period for both January – December and June – November tick collections, P = 0.0029 and P = 0.0043, respectfully as seen in Table 7. Additionally, the slope-intercept plots show an interesting trend, also broken by the 2-year lag GE2. The trends seen in the slope values in Table 8 and the plots in Appendix K involve positive slope in the Coastal zone and a negative slope in the Inland zone, with the exception of the 2-year lag GE2 drought stress for June – November adult tick collections. This latter period shows a negative slope for both the Coastal and Inland zone. Furthermore, the LS Means results show a trend of fluctuation of tick collections across the drought stress factors, with the Coastal zone increasing (Low to Medium) then decreasing (Medium to High) in drought stress and the Inland zone is opposite, with a decrease (Low to Medium) and then an increase (Medium to High) in drought stress. There are two exceptions to these trends; one is a constant decrease (Low to Medium to High) for the inland during the January – December tick collection period. The other is a constant decrease (Low to Medium to High) for both tick collection periods as the amount (area) of 2-year lag GE2 drought stress increases, Table 9. Overall, these plots indicate the increase or decrease in collections is fairly slow or steady without major swings either way as related to the amount of GE2 drought stress, although the 2-year lag GE2 drought stress consistently goes against the results from the other two lag periods.

The second issue of noise should be considered in regards to the data when compared with the phenology of *A. maculatum*. The tick collection graph, Appendix A-3, depicts the phenology of adult Gulf Coast ticks fairly accurately in regards to when adults tend to be on hosts and available for collection. However, it also shows the lack of adult activity, seen by the lack of collections roughly October – May, which encompasses the peak nymphal activity period, October – March, leading into the next adult generation. This lower season period of activity for adult ticks, combined with the dynamic nature of drought, seen in the plots in Appendix G, might contribute to the noise in the model and could possibly explain the positive slope seen in the Coastal zone during these shorter drought stress lag periods. Likewise, the Gulf Coast tick's ability to survive and even benefit from high moisture environments, by extracting moisture from sub-saturated air, could also account for the positive slope seen in the Coastal zone but not the Inland zone collections, over the short term.

Individually these results might seem random or scattered, however when taken together and in the context of *A. maculatum* producing only one generation per year and whose adults can be found in the environment up two generations, they start to come together in a meaningful way. Preliminary runs of this model, looked at individual drought stress levels across the lag periods, and showed no discernable differences leading to the focus on the 2-year lag GE2 drought stress across the different collection periods. Disadvantages to the shorter drought stress lag periods could include 1) the immature period, October to March, being a very narrow window and 2) much of the year has already played out for the current tick generation with only a 1-year lag. The 2-year lag however, captures the affect of drought stress on two nymphal cycles and continues into a second generation of adults. Looking at drought stress >2 years would not be relevant because all tick generations affected by the extended drought stress period would have already perished. Because there are still many unknowns regarding the success of *A. maculatum* in hot, dry climates these results indicate there is reason to further examine the 2-year lag GE2 drought stress effects and other possible sources of noise or contributing factors in the model.

5. SUMMARY AND CONCLUSION

5.1 Summary

This study set out to provide a very broad stroke analysis of a subject that has traditionally been addressed at a micro-level and overall it has accomplished that task while highlighting a few additional areas to investigate. A key aspect or limitation for this study remains it is a retrospective study looking only at previously collected data and with no design input for the model, methodology or consistency, these aspects are inherent to many retrospective studies. The three main goals of this study were to: 1) compare Gulf Coast tick population changes from 2000-2014 between two adjacent geographic areas of Texas; defined as inland and coastal zones, 2) compare drought stress from 2000-2014 between the coastal and inland Gulf Coast tick areas and 3) test whether trends in Gulf Coast tick population changes can be explained by corresponding changes in drought stress.

The analysis of the *A. maculatum* tick collection data indicates there is a difference between the Coastal and Inland zone. Actual tick collections are far greater in the Coastal zone (86%) as opposed to the Inland zone (14%) across the study area, despite the Inland zone being almost 10,000 square miles larger than the Coastal zone. However, the host species distribution was comparable with bovines making up 98% (7794) and 89% (1107), between the Coastal and Inland zones, respectfully and canines coming in second with 1% (70) and 6% (79), respectfully. While analysis only showed interaction between location and year for the January – December period (P = 0.0328), not the June – November period, location was highly significant (P = <0.0001) for the shorter, latter period. Overall, the analysis showed there is a difference in the tick collection between the Coastal and Inland zones of the study area. There could be additional factors, not explored in this study that contribute to the difference or biases not fully elucidated concerning the nature of the collection procedure. A very important component was the identification of four *A. maculatum* nymphs, accompanying adult tick collections from ruminants, other than bovine. This finding during peak nymphal activity months, October – March, is key to understanding the potential role *A. maculatum* could play in vectoring important pathogens, to be discussed later.

The analysis of the U.S. Drought Monitor data was a little less conclusive with highly significant interaction (P = <0.0001) for Year * Location across all seven drought stress levels. However, the LS Means plots put this into perspective showing the dynamic and often cyclic nature of drought stress in the study area. It also indicated periods of little to no drought stress during a key period for nymph activity, October – March, although that too was somewhat dynamic across the 15-year study period. The consistent flux of the drought in time and space, in both the Coastal and Inland zones, most likely plays a role in the results seen in the last objective. However, alone the analysis provided very few answers other than characterization and visualization of the constant shifting nature of drought stress.

The overarching question of whether changes in drought stress can elucidate population changes in the Gulf Coast tick population is promising but not definitive. The main idea was to explore if the USDM, with its incorporation of advances in remote sensing technology combined with local and regional climate inputs, could provide enough information to forego direct climate monitoring in order to foresee changes of Gulf Coast tick populations. From a big picture perspective, the analysis indicated the most useful model to pursue is the affect of a 2-year lag of GE2 drought stress during the peak adult activity time period of June – November. While location was highly significant (P = <0.0001) for both Coastal and Inland zones and across the October – March, 1-year lag (October – September) and 2-year lag (October – September) drought stress periods, only the 2-year lag GE2 drought stress referenced above showed significance across all analyses.

The most important factor in this study goes back to the phenology of the Gulf Coast tick and its ability or requirement to sustain itself in an off-host environment devoid of nutrition or moisture for extended periods of time between life stages. This situation can subject the tick to the risks of desiccation in an unfavorable or a long-term drought-stressed environment. Current and past literature (Needham and Teel 1991a, Sigal et al. 1991b, Yoder and Spielman 1992a) supports *A. maculatum* extracting efficient moisture from sub-saturated air to avoid desiccation. However, Teel and Strey (unpublished data) shows both *A. maculatum* survival for >400 days in canopied habitats, irrigated and non-irrigated and loss of survivorship (<25 days) in unshaded, non-irrigated open grass habitat. Their study examined micro-level conditions that were indicative of both drought and protective conditions.

The 2-year lag GE2 drought stress level for the June – November period encompasses the extended survivorship seen in their work and appears to provide a favorable means by which to explore methods to gauge tick population changes. This analysis combination alone however may not be able to explain all the noise perceived in this study and most likely will require additional monitoring methods either to bolster or disprove current results through expansion and replication.

One assumption of this study is that the collections are representative of the tick population and the drought clusters account for drought stress levels on the ticks collected at the auction house. We know by looking at the LS Means plots for drought stress that it is very dynamic and variable across the drought stress levels for any given area. However, TAHC personnel have vast experience in monitoring the cattle influxes and if their feedback is that >90% of the cattle come from the auction county or an adjacent county, then the summed weighted area drought clusters appear to be a reliable means to judge drought stress impact. Even direct climate variable monitoring would only seem applicable to a small geographical area without the support of larger-scale input from remote sensing. Likewise, we know that while the collection centers were deemed "reliable" due to servicing primarily cattle and having consistent manpower and collection submission, other outside factors are involved that can add noise or under or overinflate collection numbers. It is the opinion of this researcher that, if anything, the current tick collections greatly under estimate the tick population. Based on this belief and that any more rigorous or restrictive surveillance methods would most likely be very cost and manpower prohibitive, this collection method is viewed as the best program available for the allotted resources.

One important, albeit very small piece to the data set, was the identification of the four *A. maculatum* nymphs during the October – March period. As mentioned in the introduction, *A. maculatum* has demonstrated vector potential in the laboratory to transmit *E. ruminantium*, the pathogen causing Heartwater (Mahan et al. 2000). The question is if *A. maculatum* also has vector capacity, the ability of the immature stage to pickup the pathogen from an infected host, pass it to the adult tick stage (transstadial transmission) and then the adult tick transmits it to a pathogen-free host in the field. Ruminants, to include cervids (Yabsley et al. 2008, Kasari et al. 2010), are susceptible to *Ehrlichia ruminantium* and finding nymphs feeding on adult cervids (Ketchum et al. 2005) is an important observation worth noting. We have not and are not inferring they have vector capacity for *E. ruminantium*. However, nymphs predominantly feed on small rodents or ground-dwelling birds (Teel et al. 2010), who have not been shown to carry *E. ruminantium*, so to find them feeding on a host who could be infected with the pathogen must be noted.

While this model may not explain everything, it does give strong indication there is a difference in both tick collection counts and drought stress levels based on the Coastal and Inland zones. The results provide more evidence for continued exploration using the USDM data and focusing on the 2-year lag drought stress level for June – November, than it does against it. An additional possibility that was identified but not explored was that by using a "cluster" analysis model, ticks from all adjacent counties to the center were "lost" or not counted to avoid double counting. The center counties were chosen because they were deemed to have the auction/s servicing the most cattle and consistent coverage and manpower. However, since collections could and did come from 125 out of 148 counties in the study area an assessment by individual counties at some point in time may be warranted to ascertain the impact of the lost tick collections by cluster analysis. All of the data exists to perform the analysis, but careful consideration of the model would be required before proceeding.

5.2 Conclusion

This study had several limitations, many of which have already been pointed out. The largest limitation is the inability to tailor collection methodology to help ensure data is truly representative of the current tick population. This program was instituted as part of the Cattle Fever Tick Eradication Program (CFTEP) of the U.S. and its primary role is surveillance for the cattle fever ticks, *Rhipicephalus* (*Boophilus*) *microplus* and *R*. (*B.*) *annulatus* which transmit bovine babesiosis (cattle fever) (Graham and Hourrigan 1977) along the Mexico – Texas border (Pérez de León et al. 2012). However, with that said the TAHC records are one of, if not the only database of its kind for tick surveillance, especially covering the land area of Texas. Additionally, they have graciously shared their data with us to help further our knowledge and understanding of tick populations and dynamics in Texas. To our knowledge, there is nothing else that compares or provides this breadth of information for ticks anyplace else and can only augment laboratory and field trials despite the rigidity of its methodology. A component for future work on this subject should involve data regarding the total number of cattle that move through the auction barns to assess the influence of market trends that may cycle with drought. Additionally, the number of animals to which the inspectors have an opportunity to discover ticks, for example, as a consequence of inspection and/or vaccination for brucellosis, inspectors would have hands-on opportunities to inspect animals for ticks. This information could be used as a surrogate denominator, or estimate of the number of animals "inspected" for comparison to the number of animals from which ticks were collected and submitted for identification.

Another limitation is assuming the inputs from the USDM data adequately represent the conditions experienced by the ticks at the local level. One consideration for future work would be to explore multiple local monitoring mechanisms to reinforce what the USDM is projecting at the larger scale. This sounds counter-intuitive to the idea of moving away from microhabitat monitoring, but may be necessary to validate UDSM data at some point.

As mentioned before, the model appears to have a fair amount of noise, especially regarding the opposite direction of the slope-intercept plots. One consideration is if the positive slope were actually the tick compensating for drought stress in the short term, being that the long-term 2-year lag drought stress is the point at which they are overwhelmed and survival declines. Another consideration is the influence or contributing factors from the Gulf of Mexico and the moisture it provides. Could it account for the noise or added compensation the Coastal zone appears to have over the Inland zone? This is another avenue for exploration as the model and analysis are flushed out further in future projects.

The big picture is that while tick collections may not be precise, there are instances where the trade-offs for precision over-shadow the pay-off. The end user of this type of information would most likely be a rancher and the livestock industry does not support a cost prohibitive surveillance measures that will cut into their bottom line. A rancher doesn't need to know with 100% or even 80% certainty that tick abundance will be problematic in future years. However, if this information could provide the rancher with a 40-70% idea of what to expect regarding tick burden to their herds that could allow for cost saving measures regarding acaricides, lost revenue at the sale barn due to low weight from tick burden, increased medical expenses etc. That is the ultimate goal of this project, to lay the foundation to go forward and utilize technological advances in climate monitoring for areas involving vectors of important pathogens, human and animal health and well being.

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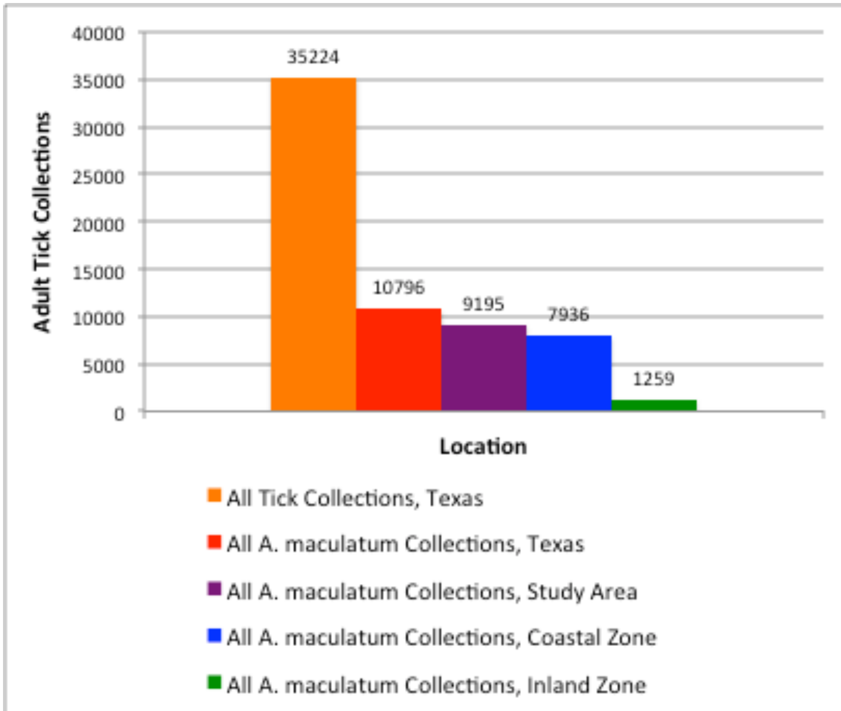
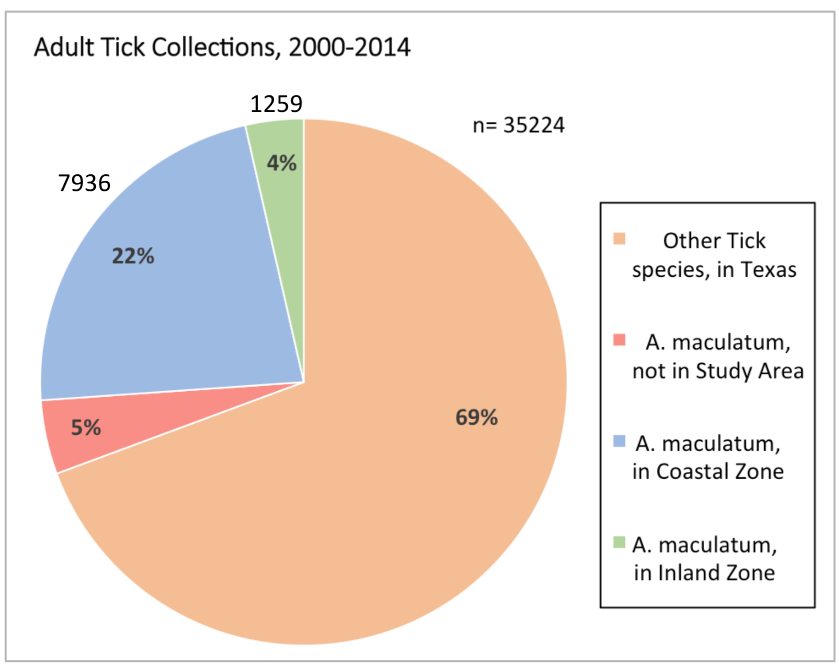
APPENDIX A

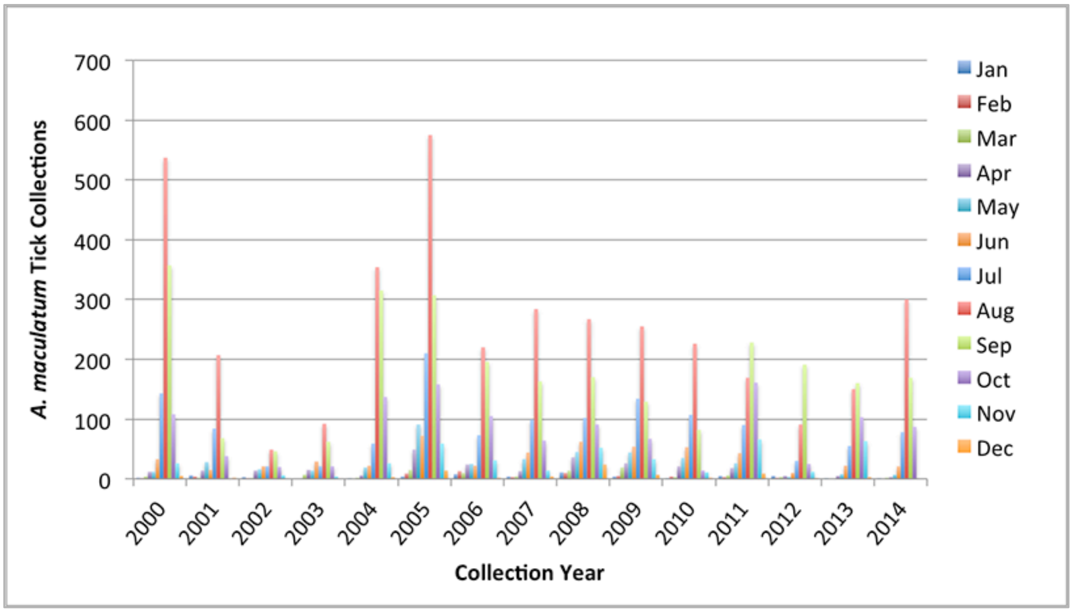
TOTAL TICK COLLECTION GRAPHS



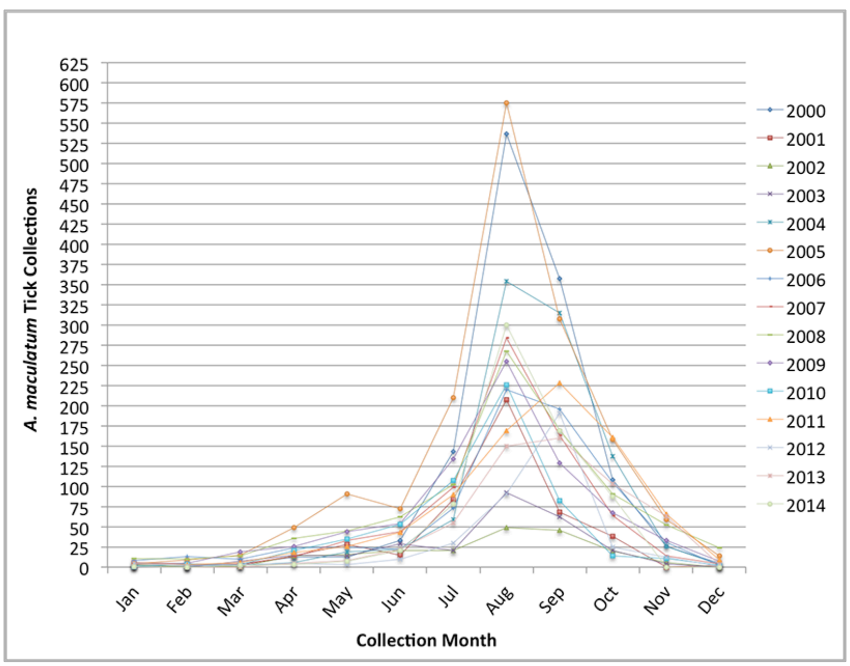
A - 1. All Adult Tick Collections by Location, 2000 – 2014.



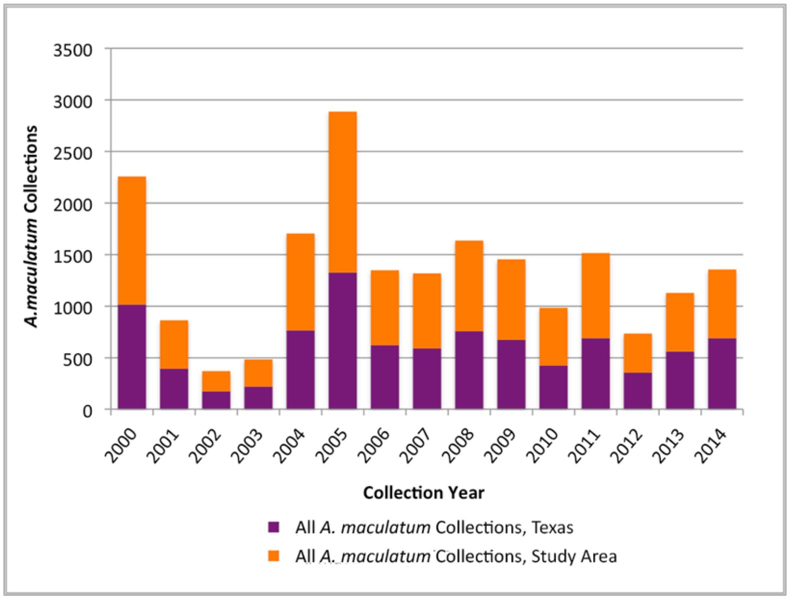
A - 2. Adult Tick Collections as a Whole, 2000 - 2014



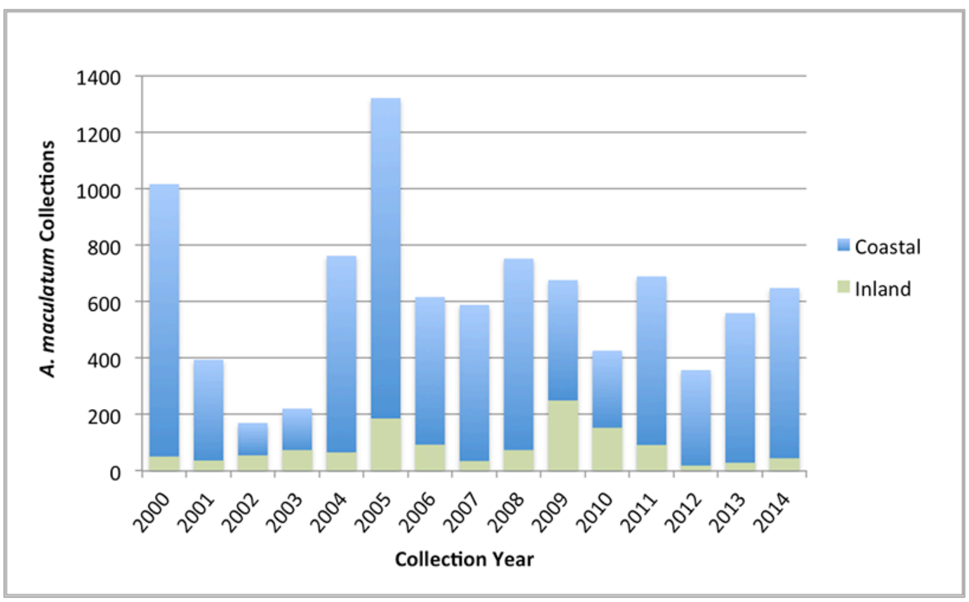
A - 3. Adult *A. maculatum* Collections by Month Across Years for All Texas Counties, 2000 – 2014



A - 4. Adult *A. maculatum* Collections by Year Across Month for All Texas Counties, 2000 – 2014



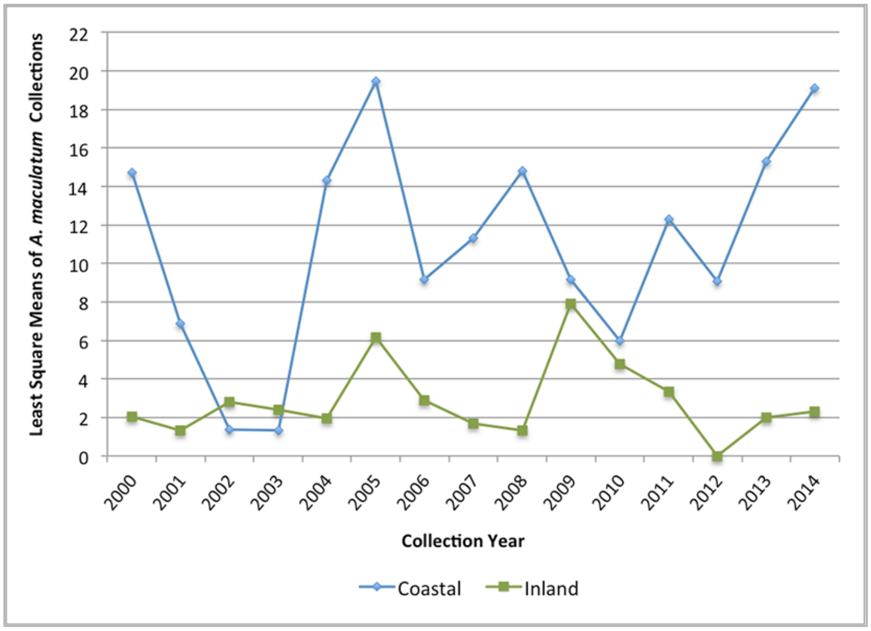
A - 5. Adult *A. maculatum* Collections by Year for All Texas Counties vs. Study Area Counties, 2000 - 2014



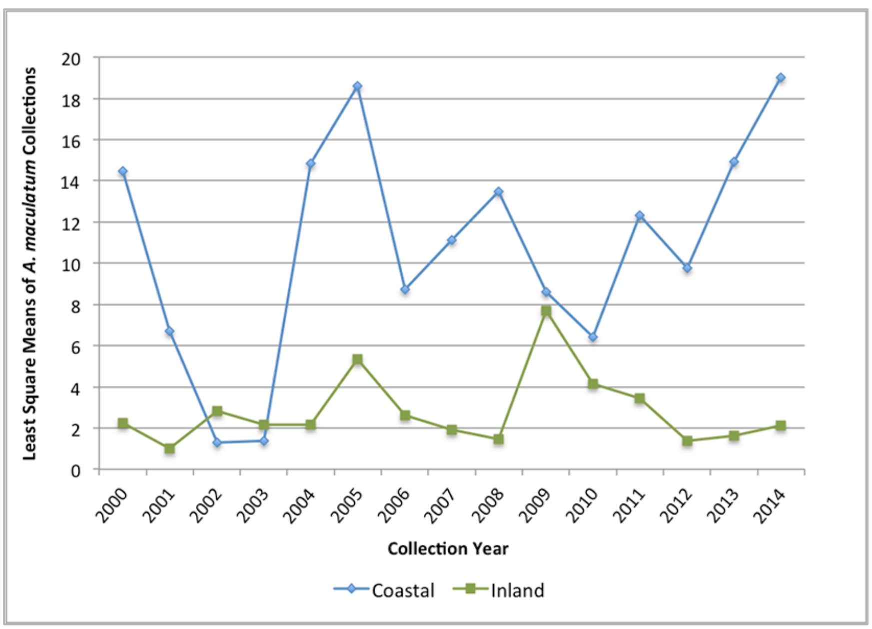
A - 6. Total *A. maculatum* Collections by Year for Coastal and Inland Study Area Counties, 2000 - 2014

APPENDIX B

LEAST SQUARE MEANS FOR TICK COLLECTIONS



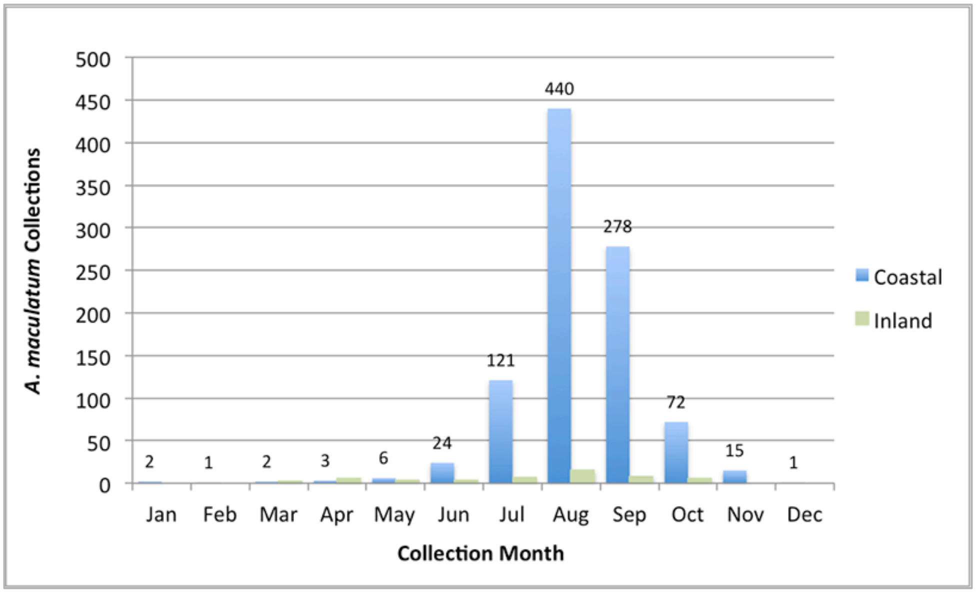
B - 1. Least Square Means of *A. maculatum* Collections by Year, January - December for Coastal and Inland Zones, 2000 - 2014



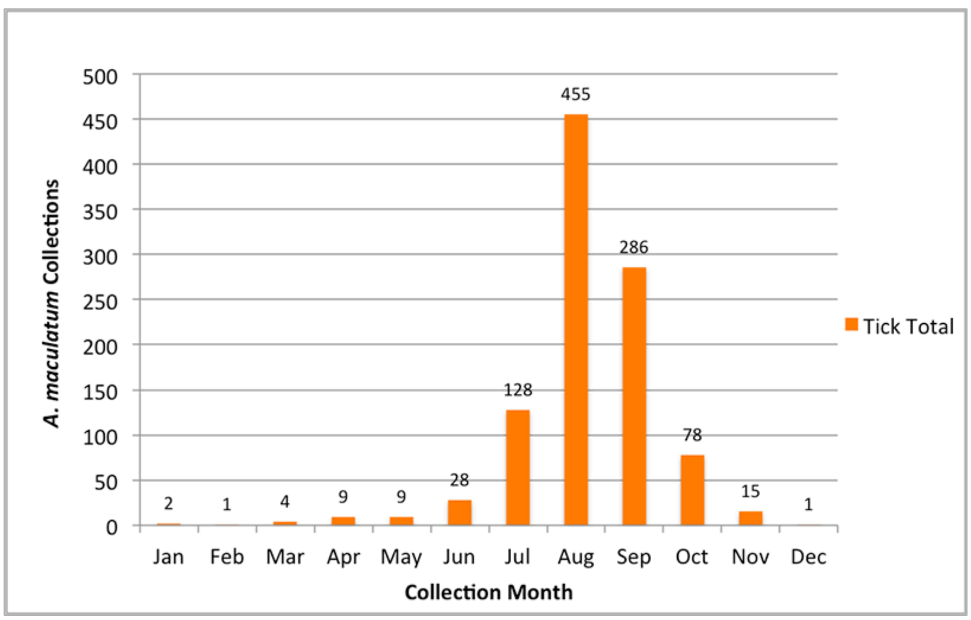
B - 2. Least Square Means of *A. maculatum* Collections By Year, June - November for Coastal and Inland Zones, 2000 - 2014

APPENDIX C

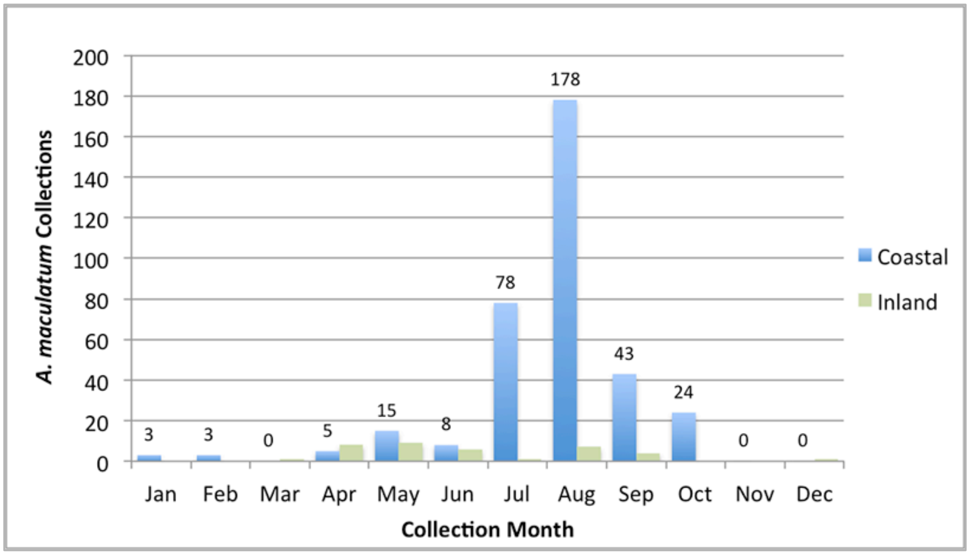
A. MACULATUM COLLECTIONS BY YEAR IN THE STUDY AREA



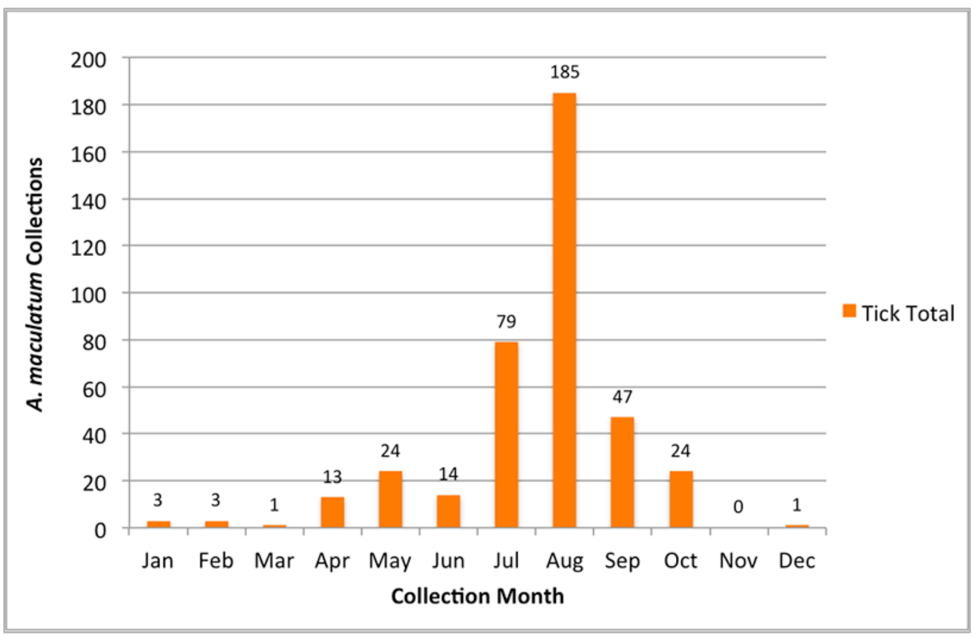
C - 1. *A. maculatum* Collections for 2000 by Month for Inland and Coastal Study Area Counties



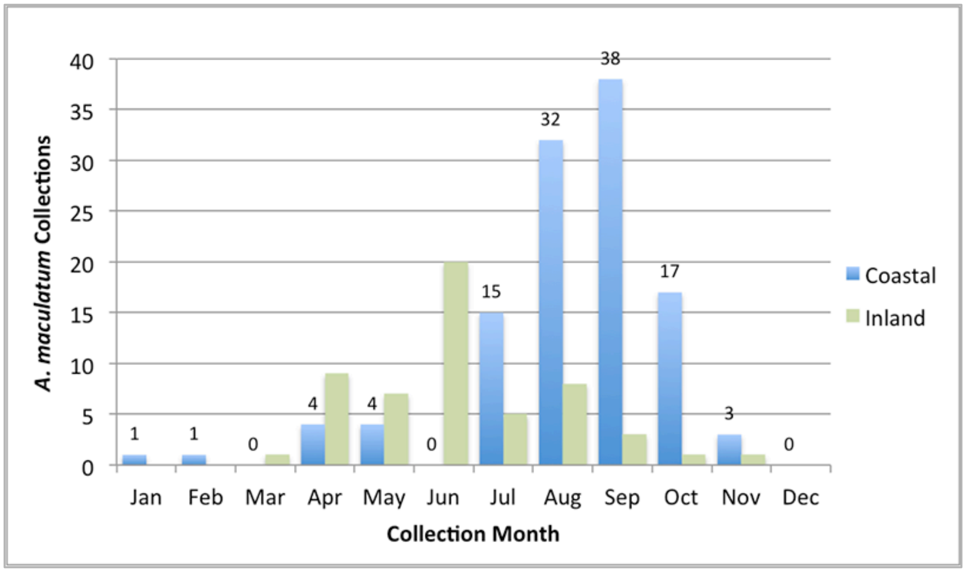
C - 2. Total A. maculatum Collections for 2000 by Month for All Counties in the Study Area



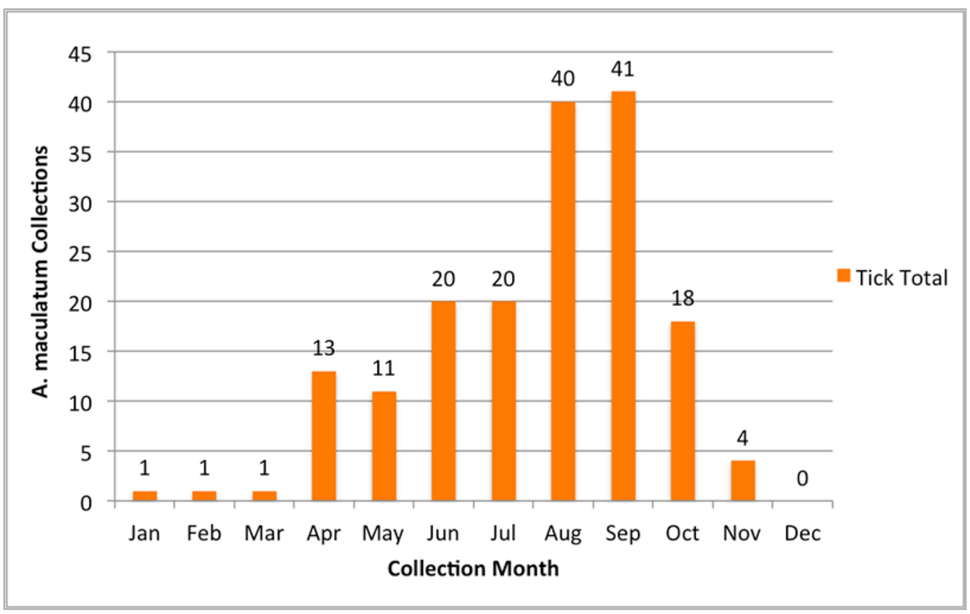
C - 3. *A. maculatum* Collections for 2001 by Month for Inland and Coastal Study Area Counties



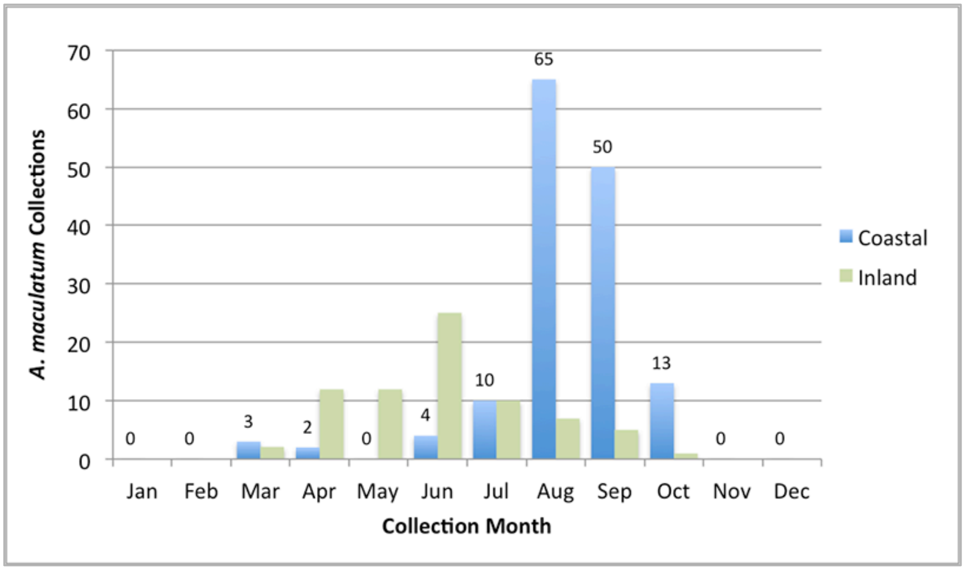
C - 4. Total *A. maculatum* Collections for 2001 by Month for All Counties in the Study Area



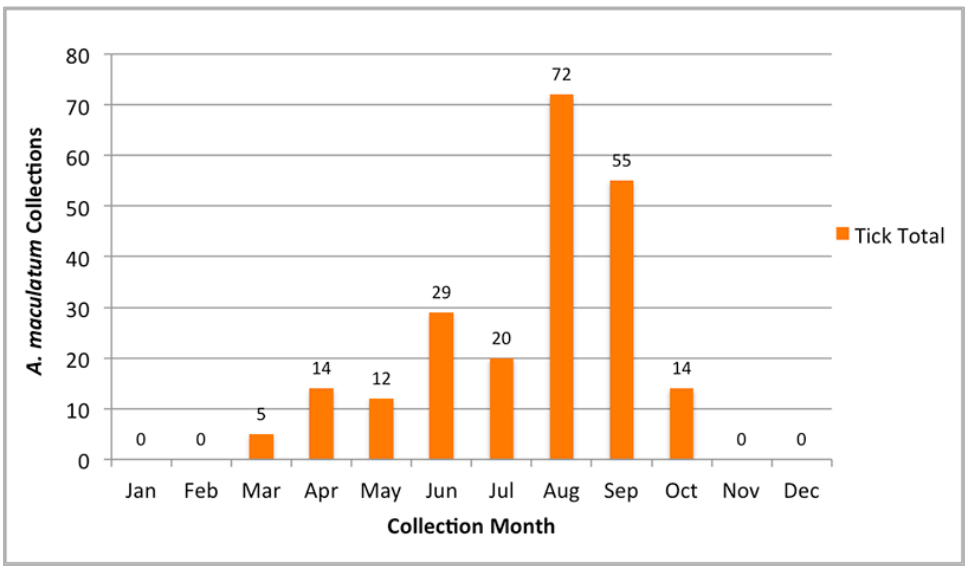
C - 5. *A. maculatum* Collections for 2002 by Month for Inland and Coastal Study Area Counties



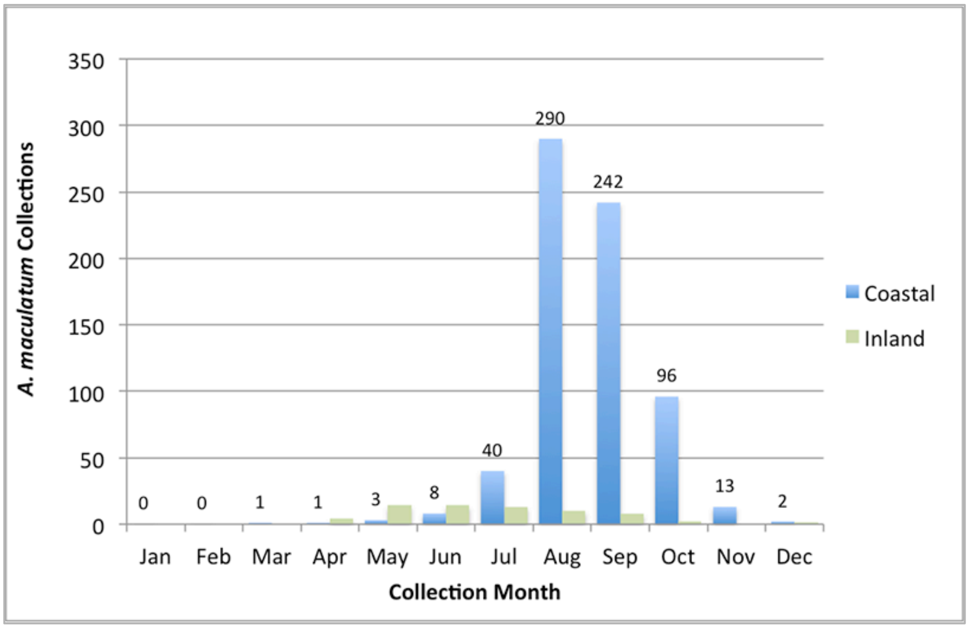
C - 6. Total *A. maculatum* Collections for 2002 by Month for All Counties in the Study Area



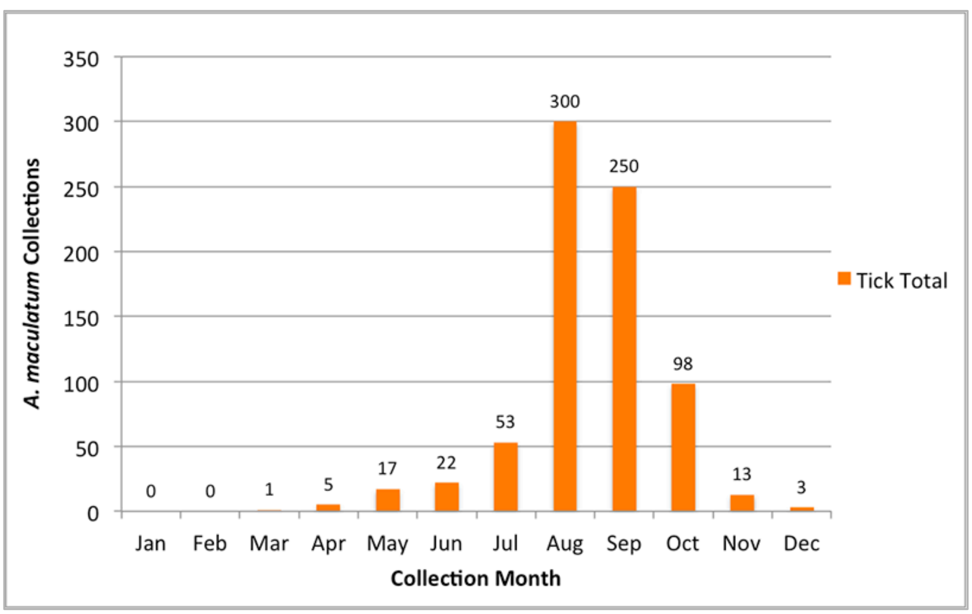
C - 7. *A. maculatum* Collections for 2003 by Month for Inland and Coastal Study Area Counties



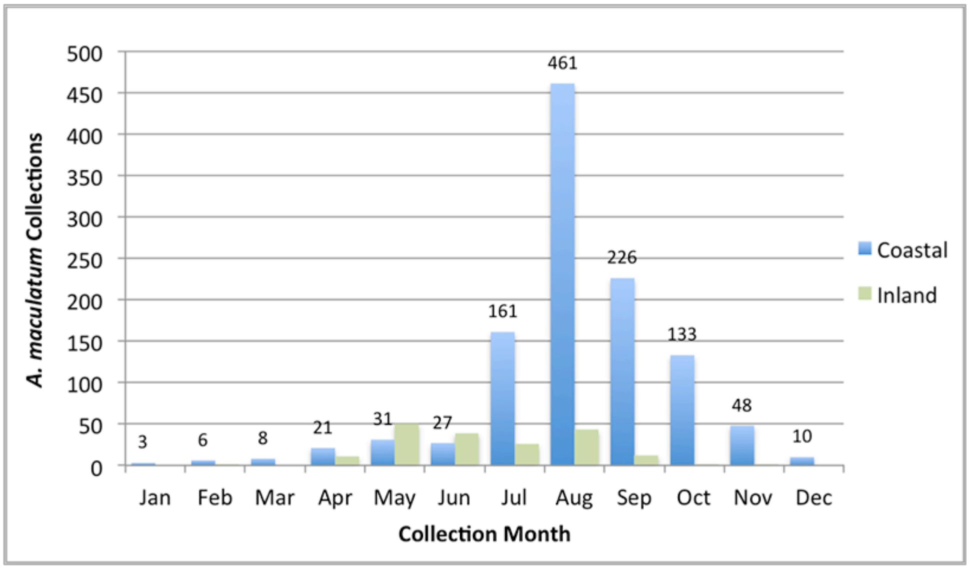
C - 8. Total *A. maculatum* Collections for 2003 by Month for All Counties in the Study Area



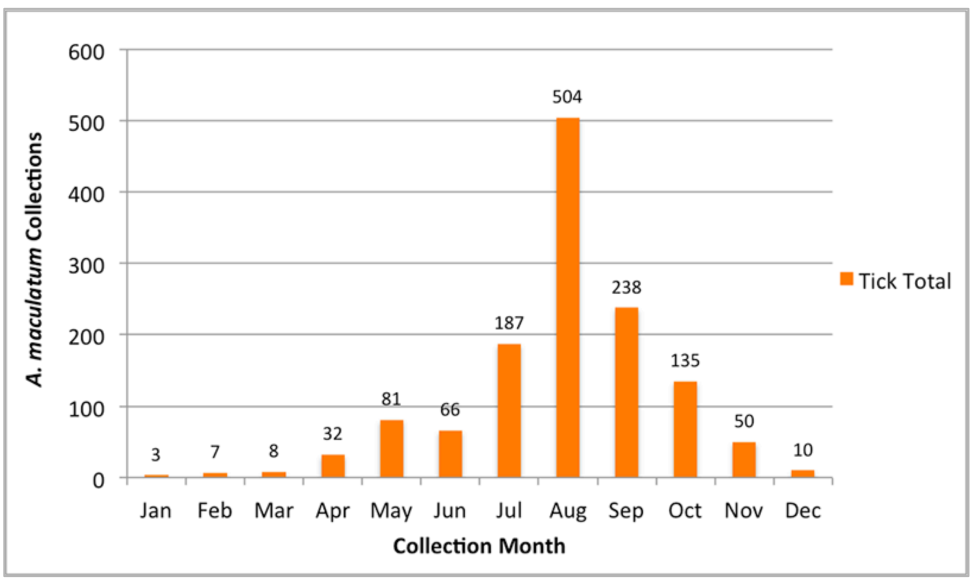
C - 9. *A. maculatum* Collections for 2004 by Month for Inland and Coastal Study Area Counties



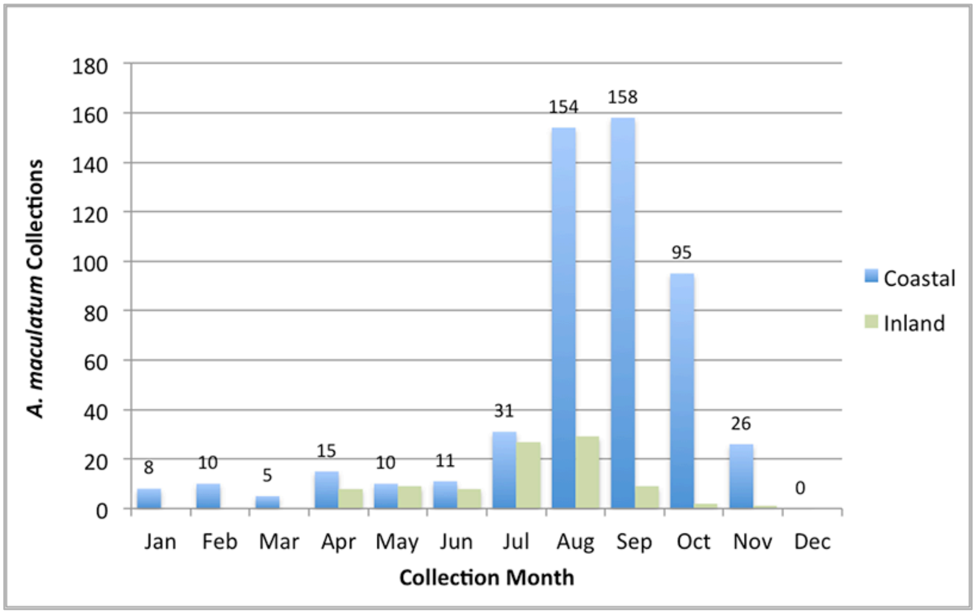
C - 10. Total *A. maculatum* Collections for 2004 by Month for All Counties in the Study Area



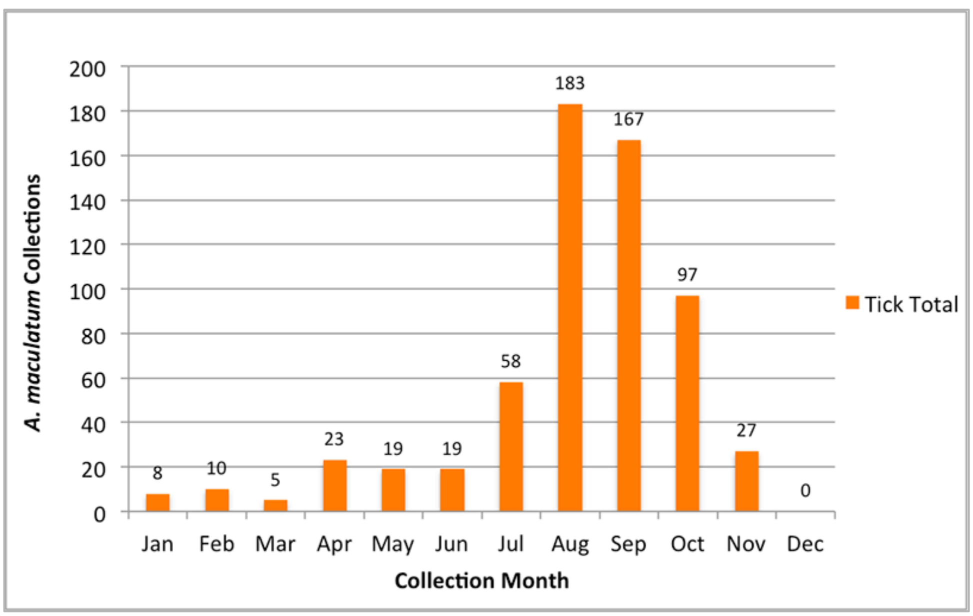
C - 11. *A. maculatum* Collections for 2005 by Month for Inland and Coastal Study Area Counties



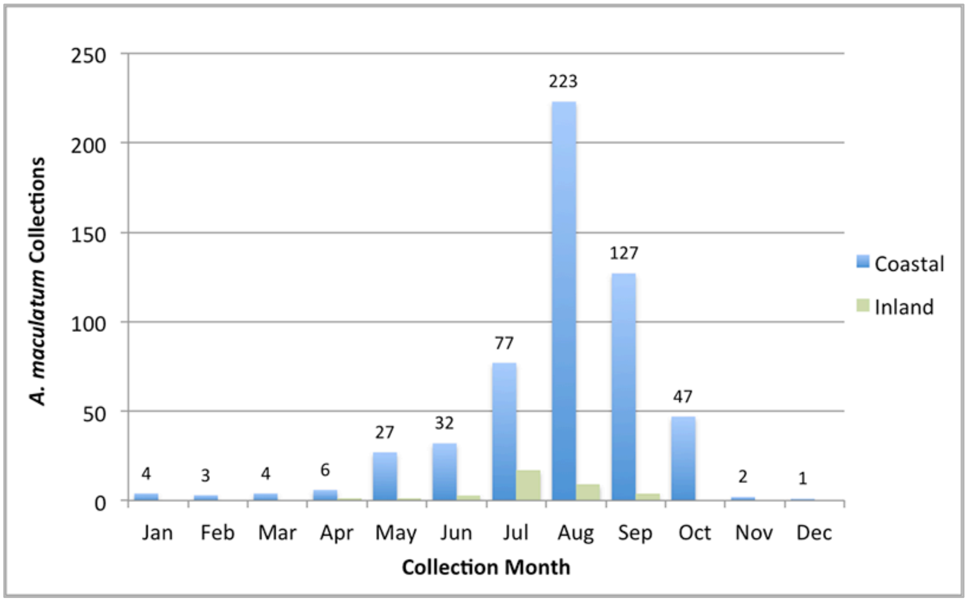
C - 12. Total *A. maculatum* Collections for 2005 by Month for All Counties in the Study Area



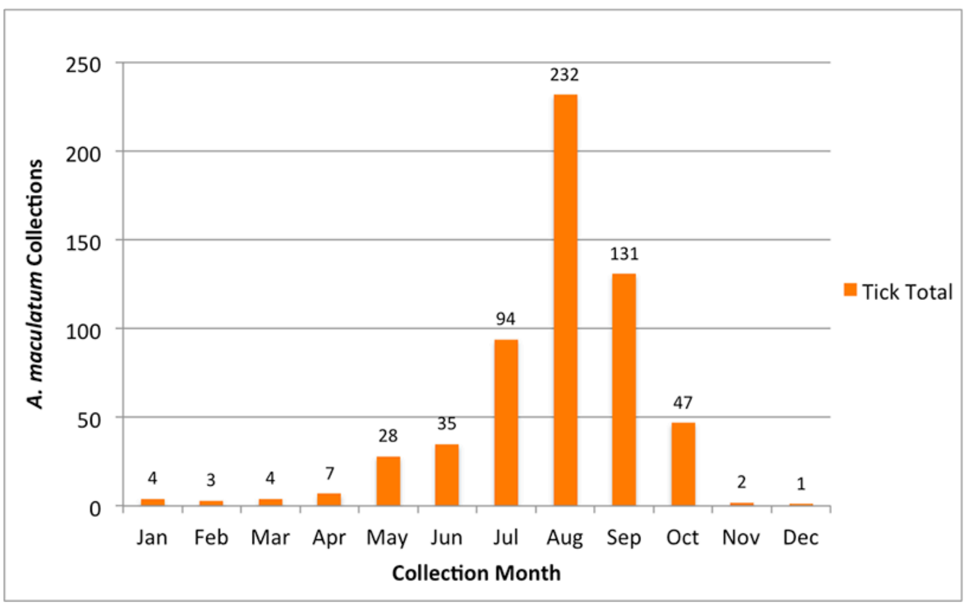
C - 13. *A. maculatum* Collections for 2006 by Month for Inland and Coastal Study Area Counties



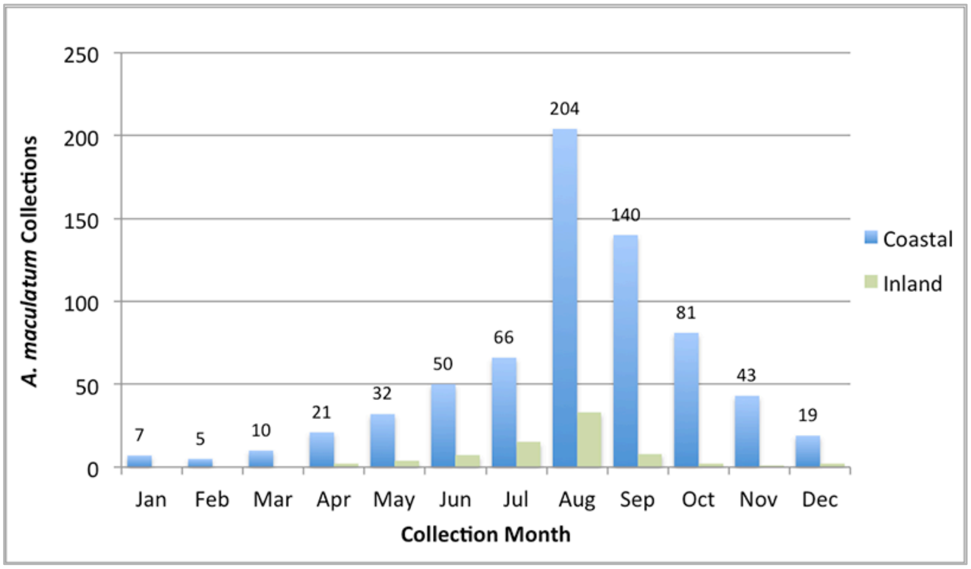
C - 14. Total *A. maculatum* Collections for 2006 by Month for All Counties in the Study Area



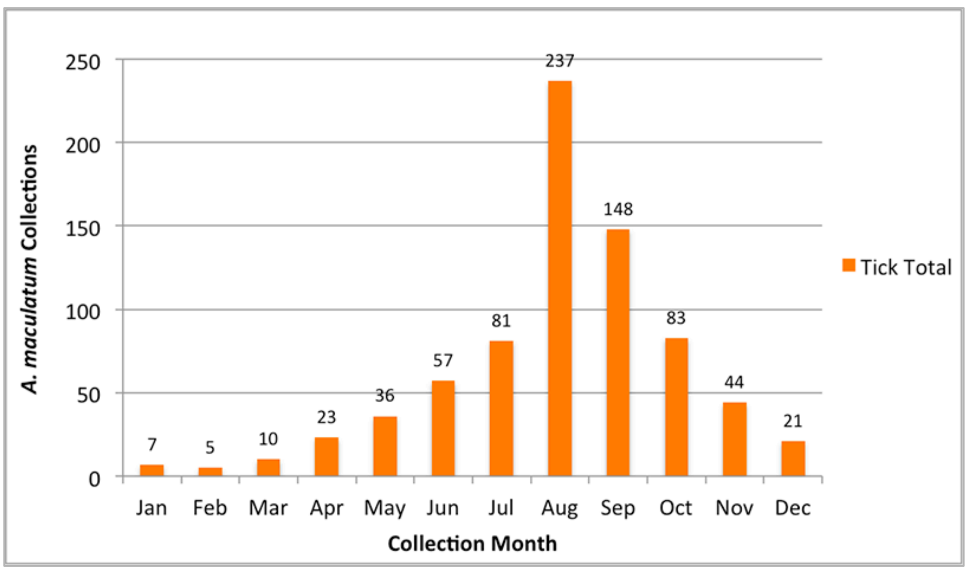
C - 15. *A. maculatum* Collections for 2007 by Month for Inland and Coastal Study Area Counties



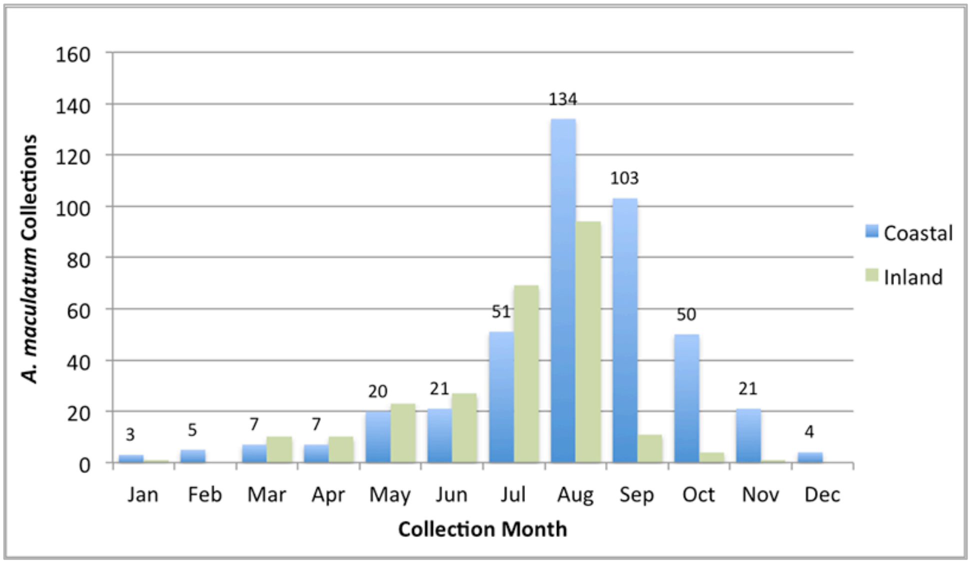
C - 16. Total *A. maculatum* Collections for 2007 by Month for All Counties in the Study Area



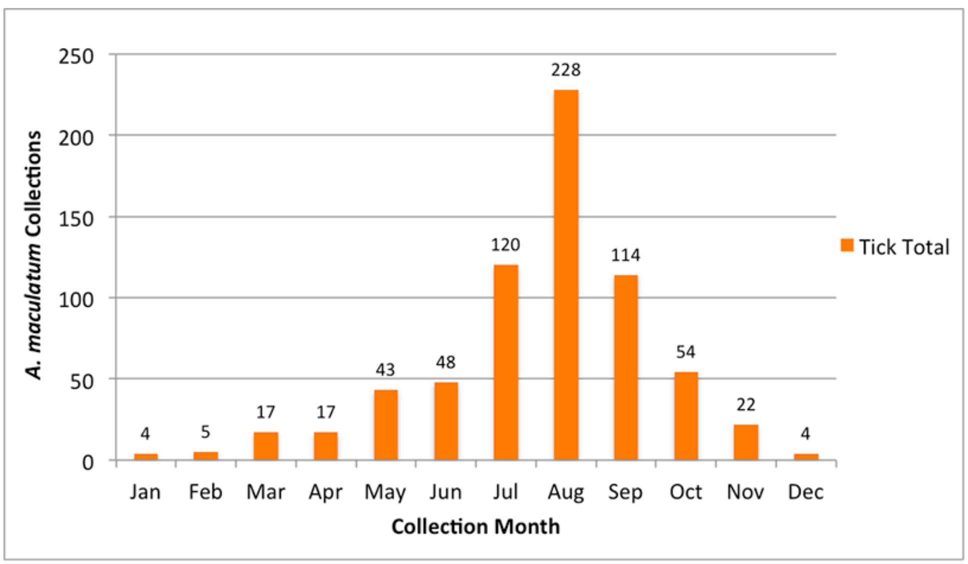
C - 17. *A. maculatum* Collections for 2008 by Month for Inland andCoastal Study Area Counties



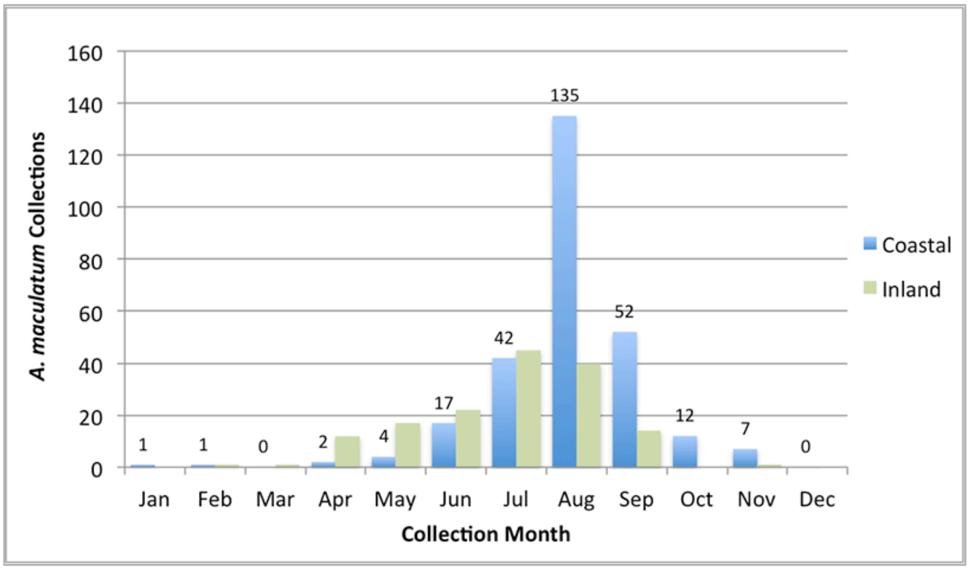
C - 18. Total *A. maculatum* Collections for 2008 by Month for All Counties in the Study Area



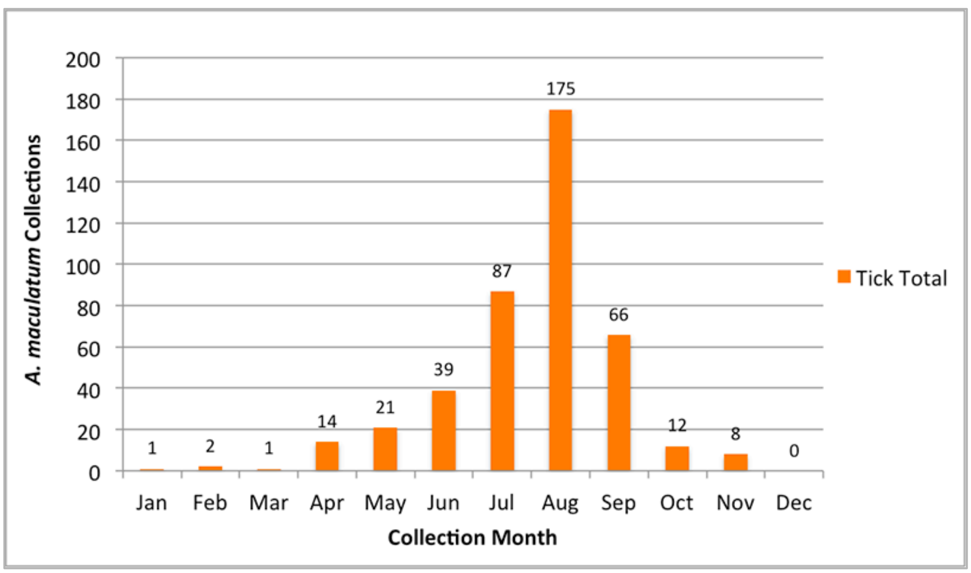
C - 19. *A. maculatum* Collections for 2009 by Month for Inland and Coastal Study Area Counties



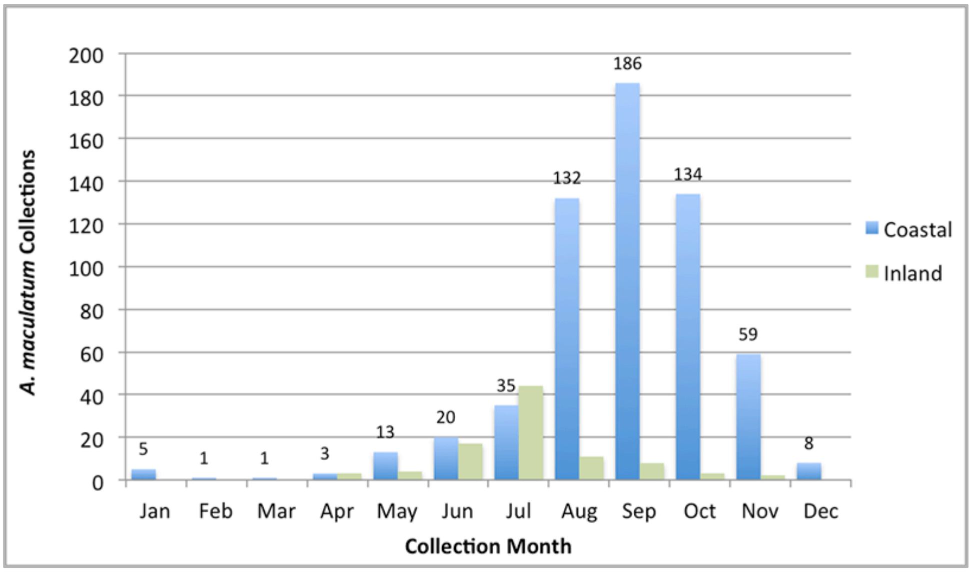
C - 20. Total *A. maculatum* Collections for 2009 by Month for All Counties in the Study Area



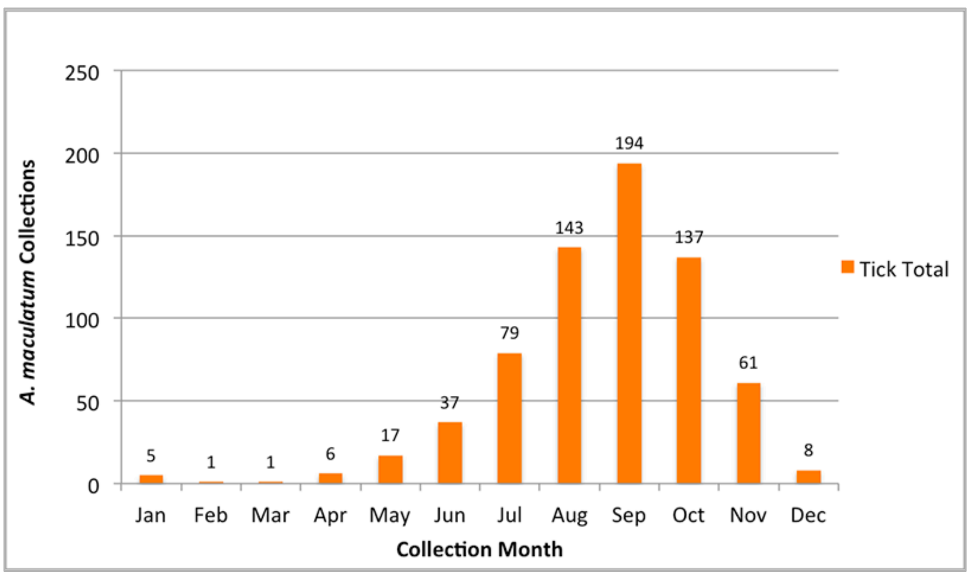
C - 21. *A. maculatum* Collections for 2010 by Month for Inland and Coastal Study Area Counties



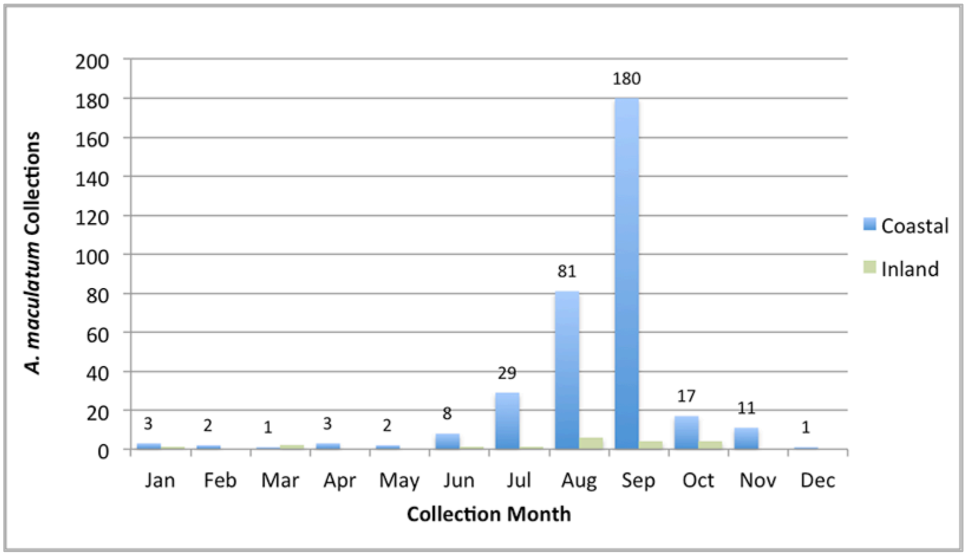
C - 22. Total *A. maculatum* Collections for 2010 by Month for All Counties in the Study Area



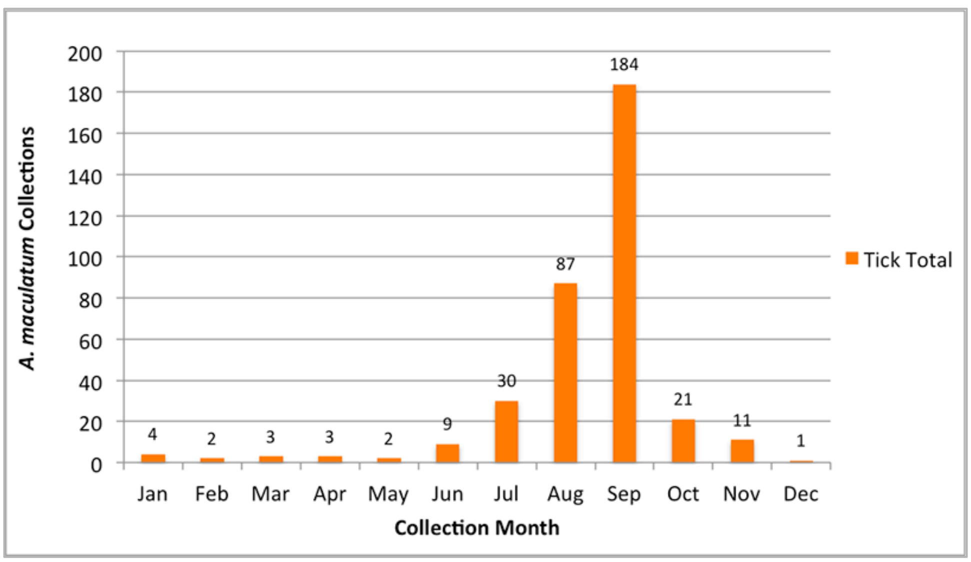
C - 23. *A. maculatum* Collections for 2011 by Month for Coastal and Inland Study Area Counties



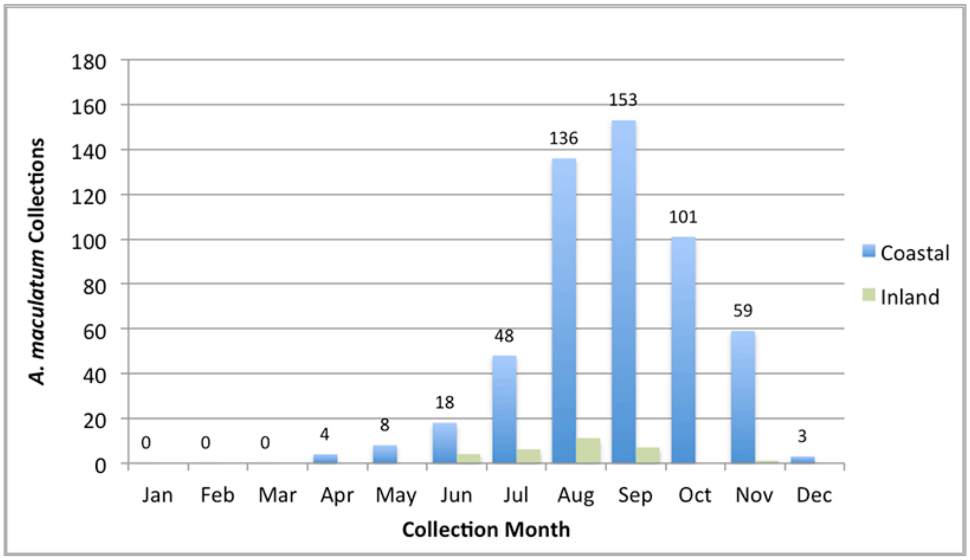
C - 24. Total *A. maculatum* Collections for 2011 by Month for All Counties in the Study Area



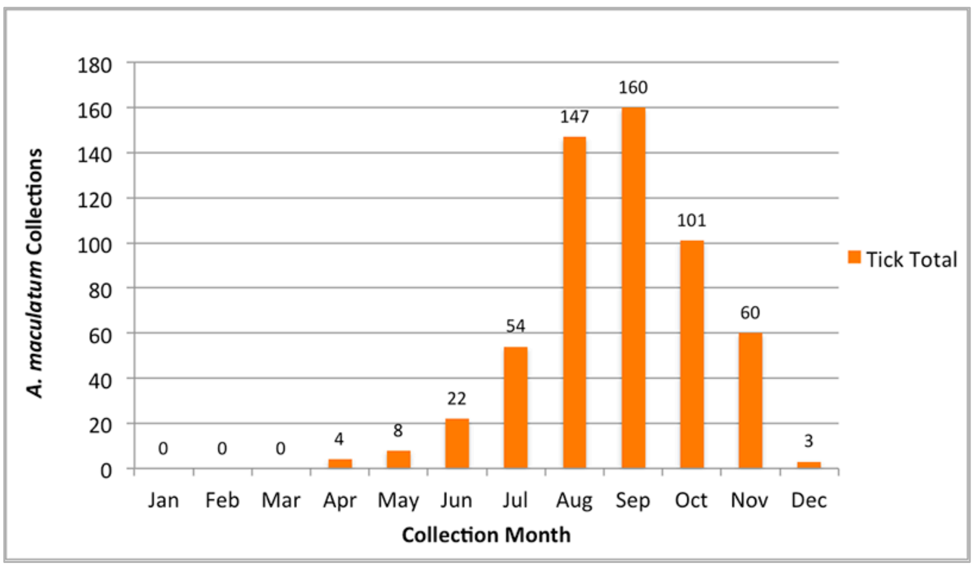
C - 25. *A. maculatum* Collections for 2012 by Month for Inland and Coastal Study Area Counties



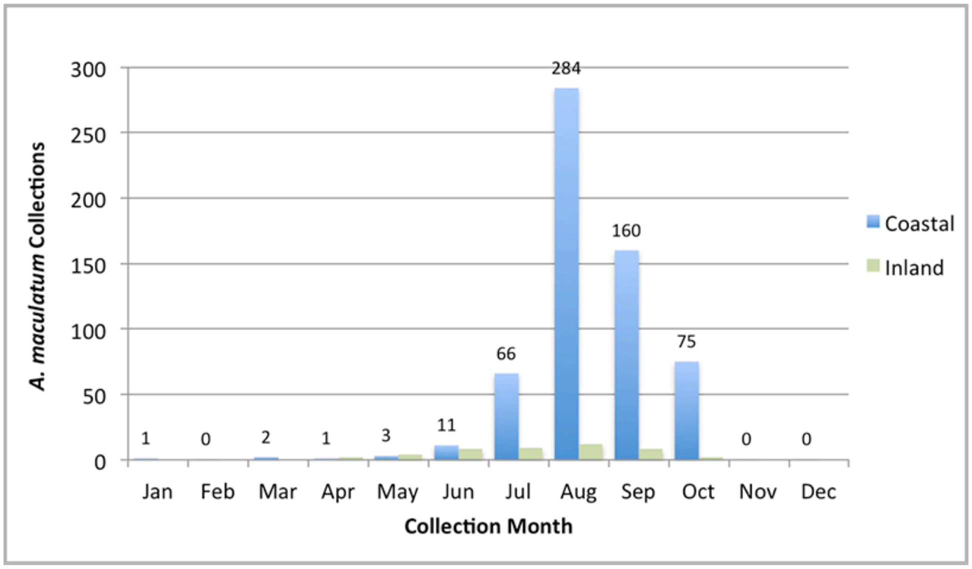
C - 26. Total *A. maculatum* Collections for 2012 by Month for All Counties in the Study Area



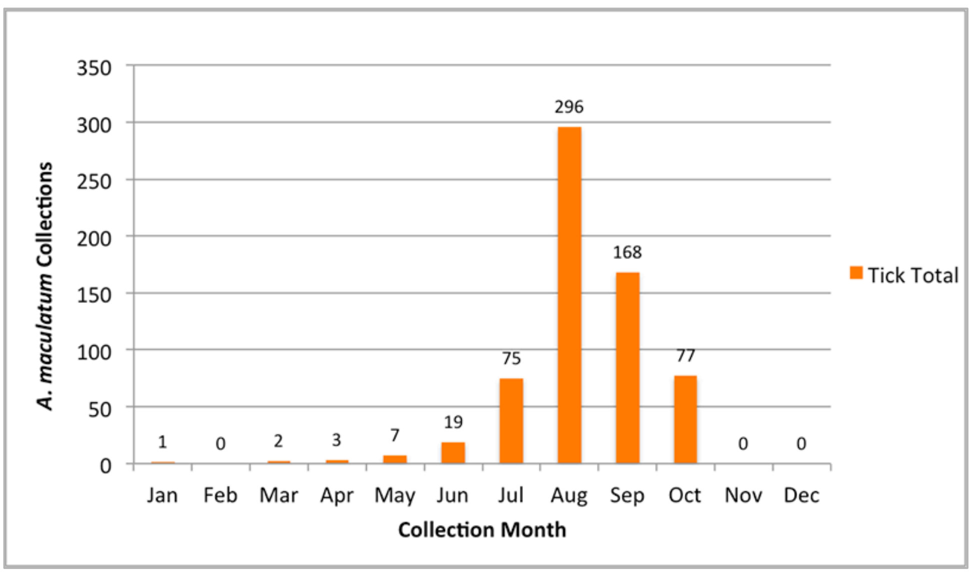
C - 27. *A. maculatum* Collections for 2013 by Month for Inland and Coastal Study Area Counties



C - 28. Total *A. maculatum* Collections for 2013 by Month for All Counties in the Study Area



C - 29. *A. maculatum* Collections for 2014 by Month for Inland and Coastal Study Area Counties



C - 30. Total *A. maculatum* Collections for 2014 by Month for All Counties in the Study Area

APPENDIX D

COLLECTION DATA BY YEAR FOR STUDY AREA

The following tables contain the yearly and monthly data used to create the graphs in Appendix C, for collections Texas wide and the Coastal (C) and Inland (I) zones of the study area.

Year	All Collections, All Texas Counties	*A. maculatum*, All Texas Counties	*A. maculatum*, Study Area Counties
2000	3614	1240	1016
2001	2441	468	394
2002	1393	198	170
2003	1569	265	221
2004	2541	944	762
2005	4946	1563	1321
2006	3744	729	616
2007	2628	729	588
2008	1828	884	752
2009	2572	777	676
2010	1584	559	426
2011	1974	824	689
2012	1205	378	357
2013	1357	570	559
2014	1828	668	648
Total Collections	**35224**	**10796**	**9195**

D - 1. Collection Data by Year for All Ticks in Texas, *A. maculatum* in Texas and *A. maculatum* in the Study Area, 2000 - 2014

Year	Coastal Collections	Inland Collections	Total Collections
2000	965	51	1016
2001	357	37	394
2002	115	55	170
2003	147	74	221
2004	696	66	762
2005	1135	186	1321
2006	523	93	616
2007	553	35	588
2008	678	74	752
2009	426	250	676
2010	273	153	426
2011	597	92	689
2012	338	19	357
2013	530	29	559
2014	603	45	648
Total Collections	**7936**	**1259**	**9195**

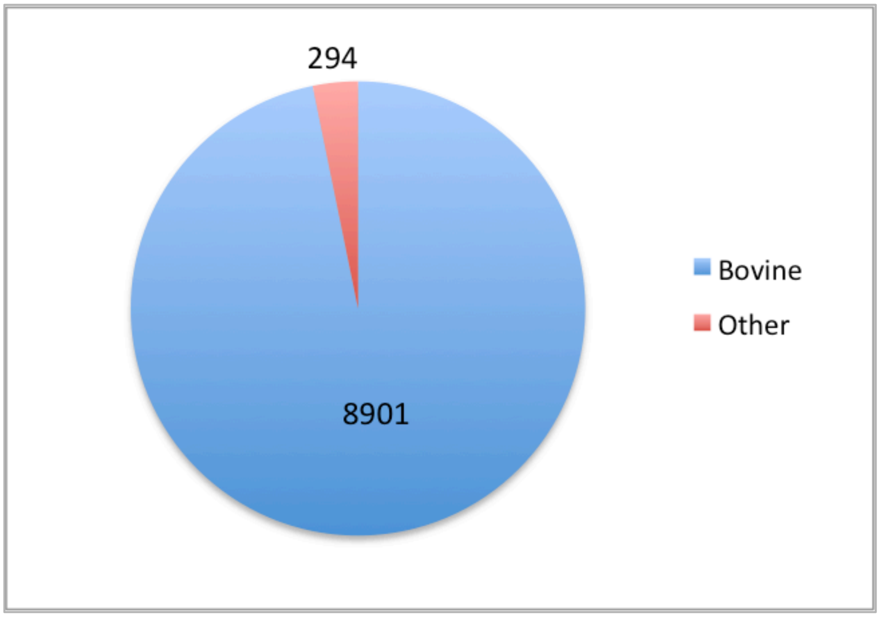
D - 2. Collection Data by Year for the Coastal and Inland Zones of the Study Area, 2000 - 2014

	2000		2001		2002		2003		2004		2005		2006		2007		2008		2008		2010		2011		2012		2013		2014		Monthly
Month	**C**	**I**	**C**	**I**	**C**	**I**	**C**	**I**	**C**	**I**	**C**	**I**	**C**	**I**	**C**	**I**	**C**	**I**	**C**	**I**	**C**	**I**	**C**	**I**	**C**	**I**	**C**	**I**	**C**	**I**	**Total**
Jan	2	0	3	0	1	0	0	0	0	0	3	0	8	0	4	0	7	0	3	1	1	0	5	0	3	1	0	0	1	0	43
Feb	1	0	3	0	1	0	0	0	0	0	6	1	10	0	3	0	5	0	5	0	1	1	1	0	2	0	0	0	0	0	40
Mar	2	2	0	1	0	1	3	2	1	0	8	0	5	0	4	0	10	0	7	10	0	1	1	0	1	2	0	0	2	0	63
Apr	3	6	5	8	4	9	2	12	1	4	21	11	15	8	6	1	21	2	7	10	2	12	3	3	3	0	4	0	1	2	186
May	6	3	15	9	4	7	0	12	3	14	31	50	10	9	27	1	32	4	20	23	4	17	13	4	2	0	8	0	3	4	335
Jun	24	4	8	6	0	20	4	25	8	14	27	39	11	8	32	3	50	7	21	27	17	22	20	17	8	1	18	4	11	8	464
Jul	121	7	78	1	15	5	10	10	40	13	161	26	31	27	77	17	66	15	51	69	42	45	35	44	29	1	48	6	66	9	1165
Aug	440	15	178	7	32	8	65	7	290	10	461	43	154	29	223	9	204	33	134	94	135	40	132	11	81	6	136	11	284	12	3284
Sep	278	8	43	4	38	3	50	5	242	8	226	12	158	9	127	4	140	8	103	11	52	14	186	8	180	4	153	7	160	8	2249
Oct	72	6	24	0	17	1	13	1	96	2	133	2	95	2	47	0	81	2	50	4	12	0	134	3	17	4	101	0	75	2	996
Nov	15	0	0	0	3	1	0	0	13	0	48	2	26	1	2	0	43	1	21	1	7	1	59	2	11	0	59	1	0	0	317
Dec	1	0	0	1	0	0	0	0	2	1	10	0	0	0	1	0	19	2	4	0	0	0	8	0	1	0	3	0	0	0	53
Yearly Total	965	51	357	37	115	55	147	74	696	66	1135	186	523	93	553	35	678	74	426	250	273	153	597	92	338	19	530	29	603	45	9195

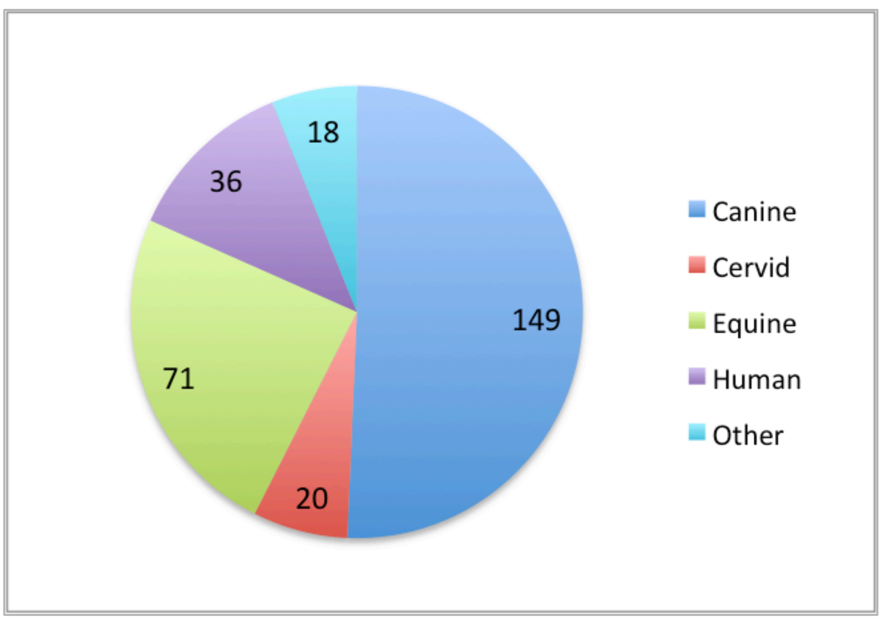
D - 3. Study Area Collection Data by Year for the Coastal and Inland Zones, 2000 - 2014

APPENDIX E

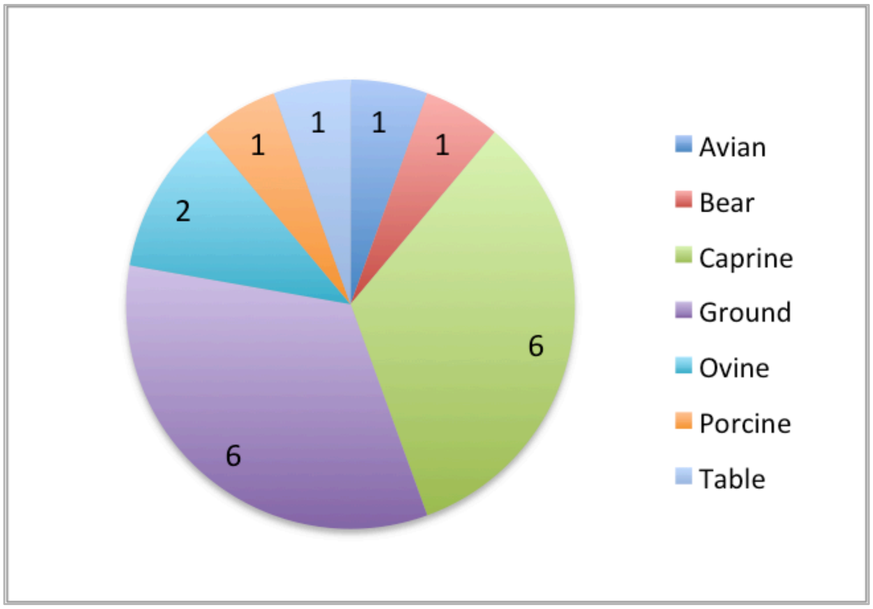
STUDY AREA COLLECTIONS BY HOST SPECIES



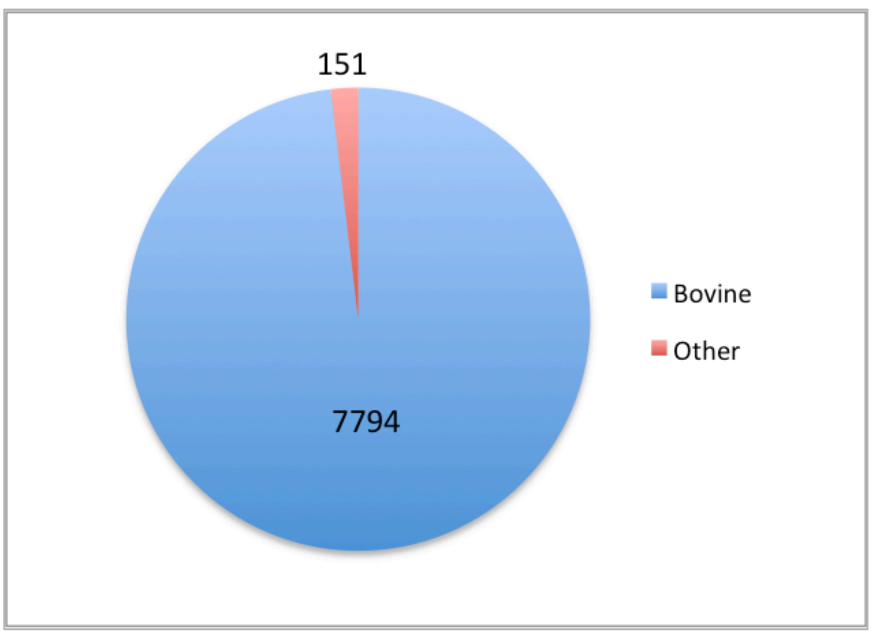
E - 1. All Study Area Collections by Host Species



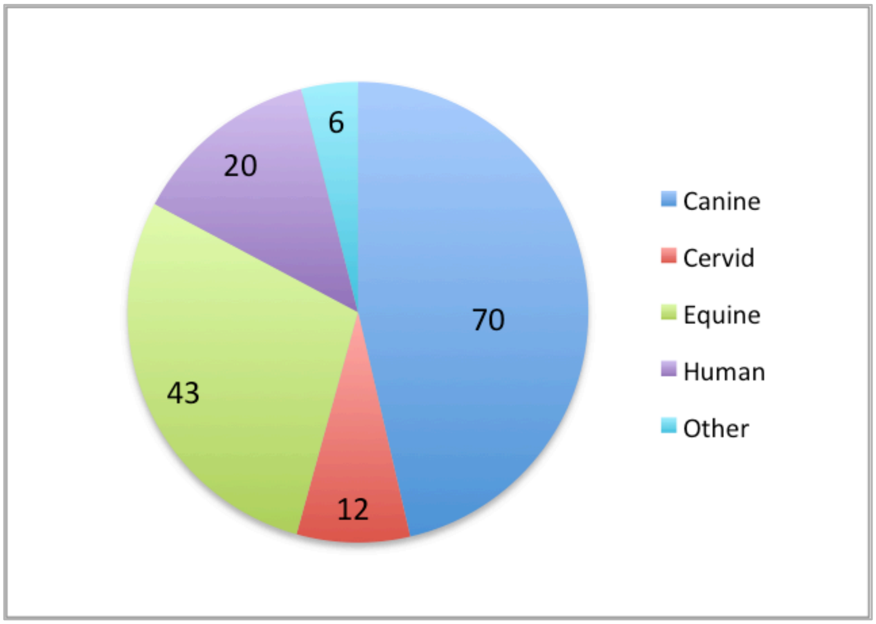
E - 2. All Study Area Collections by Host Species Without Bovine



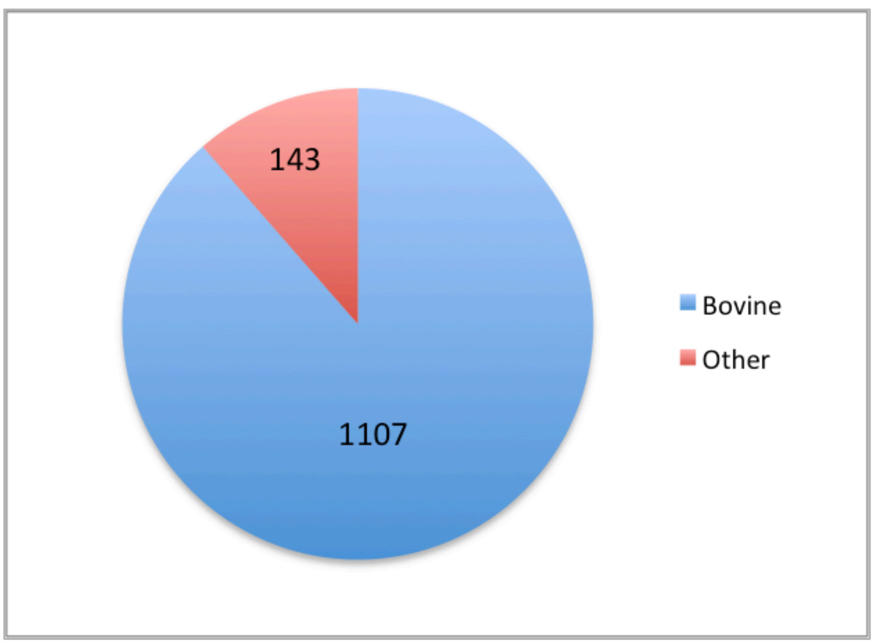
E - 3. Study Area Collections by Host Species Previously Labeled as Other in E - 2



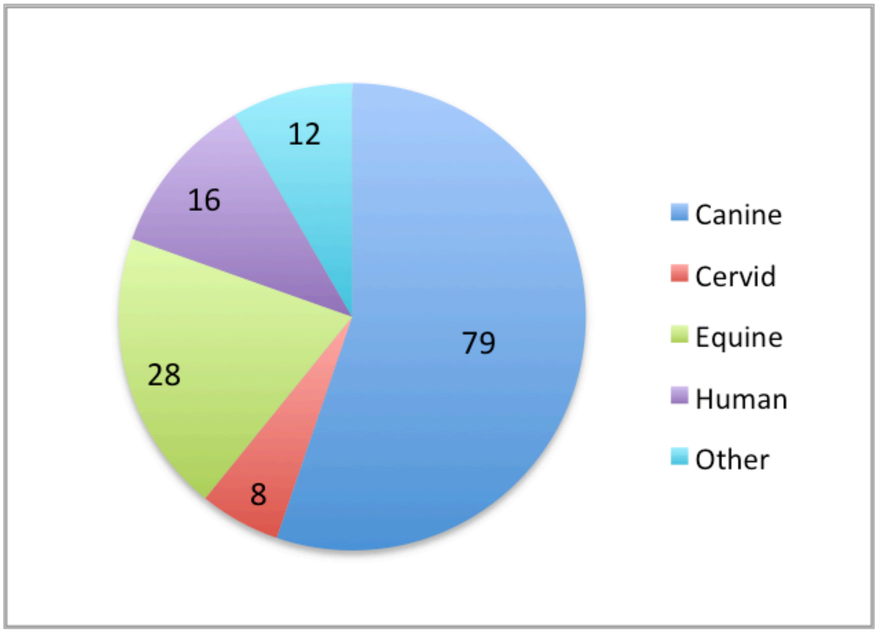
E - 4. Coastal Zone Collections by Host Species



E - 5. Coastal Zone Collections by Host Species Without Bovine



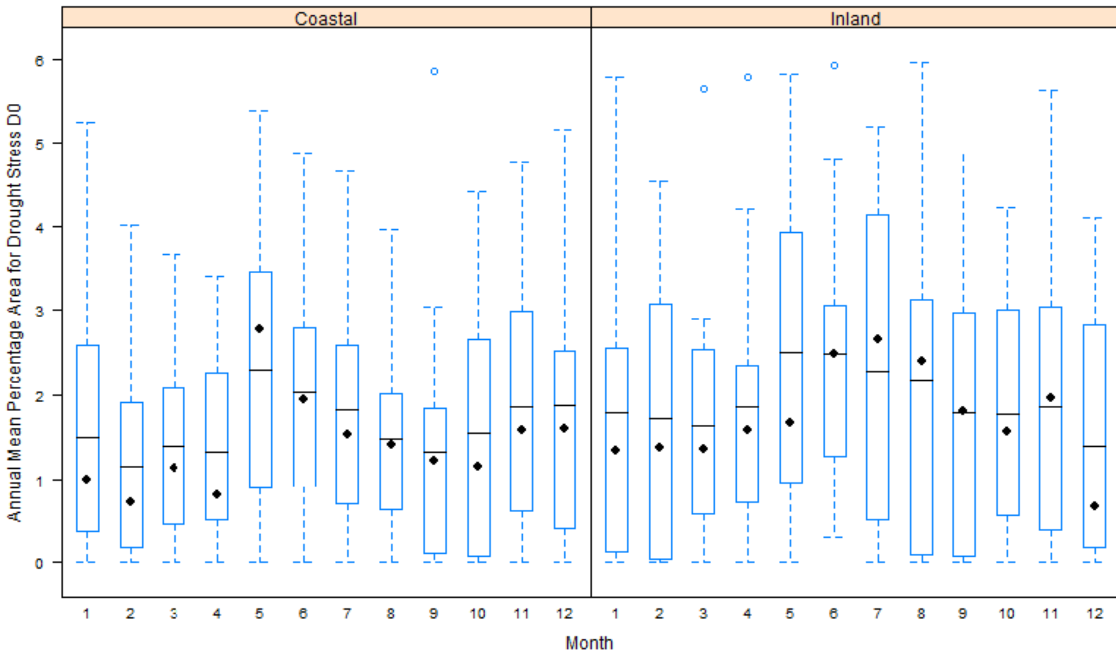
E - 6. Inland Zone Collections by Host Species



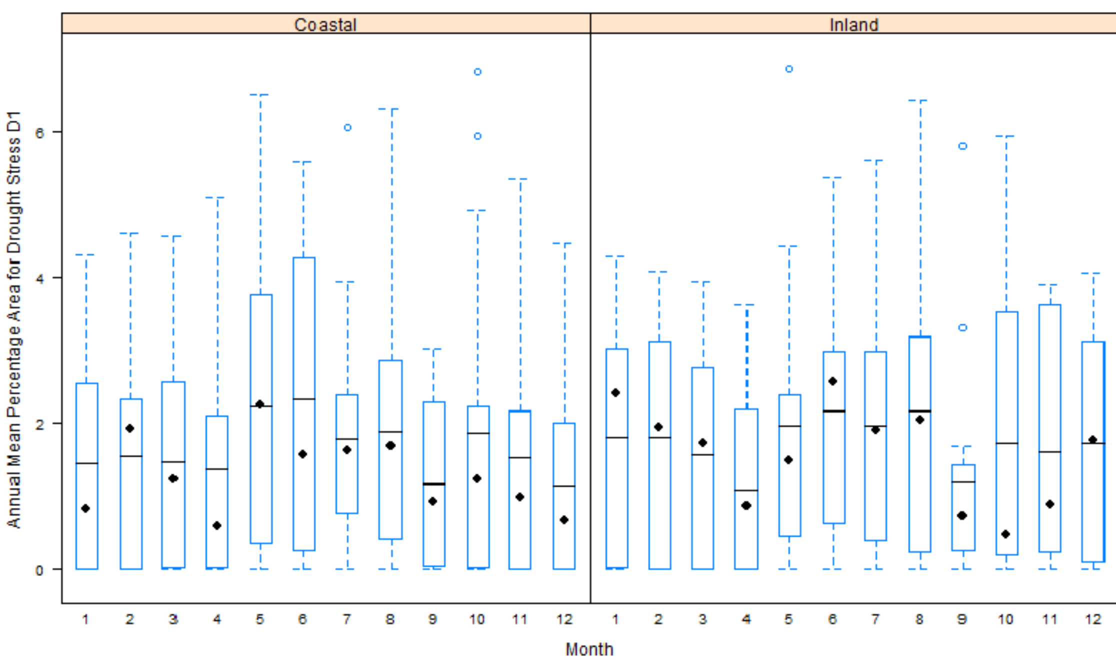
E - 7. Inland Zone Collections by Host Species Without Bovine

APPENDIX F

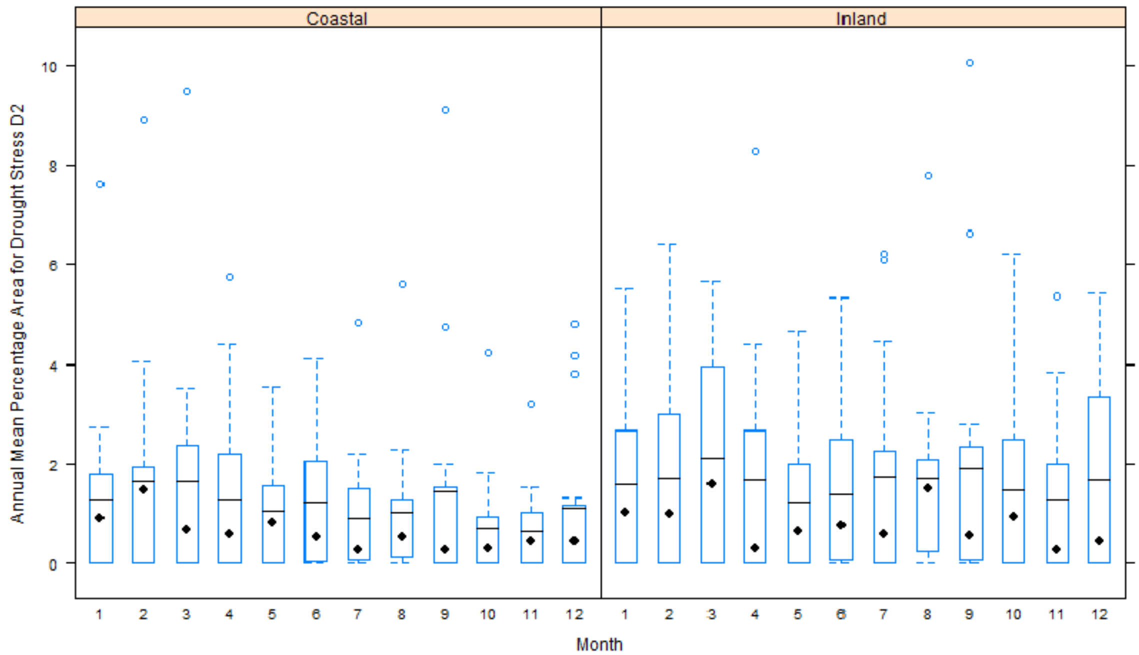
ANNUAL MEAN PERCENTAGE AREA FOR DROUGHT STRESS CATEGORIES



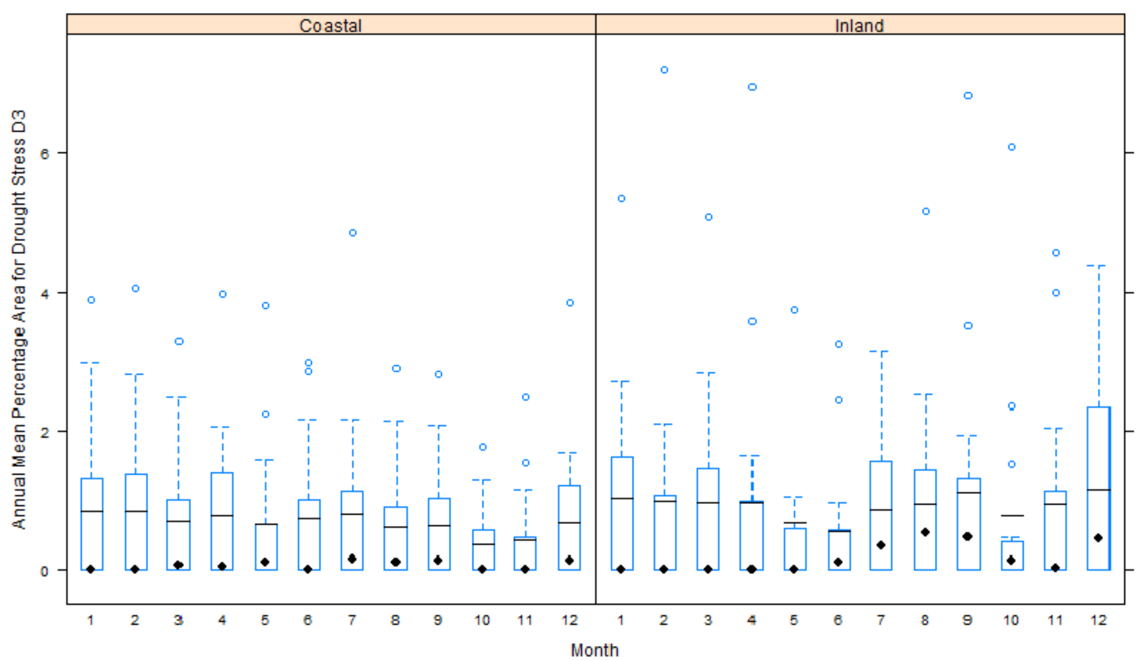
F - 1. Annual Mean Percentage Area for Drought Stress D0



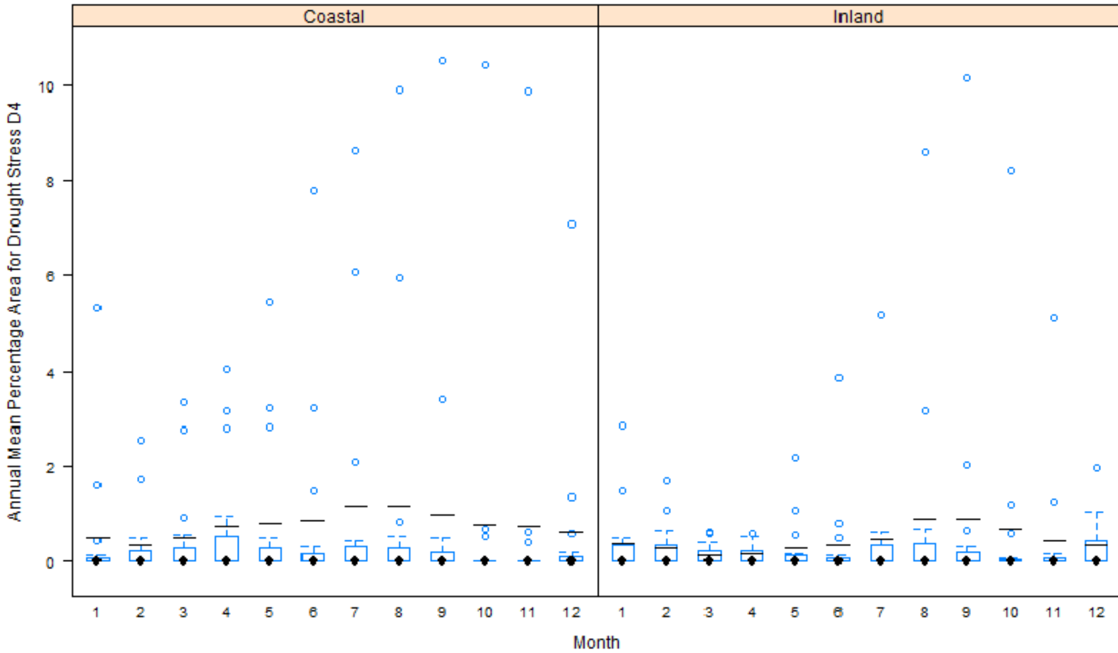
F - 2. Annual Mean Percentage Area for Drought Stress D1



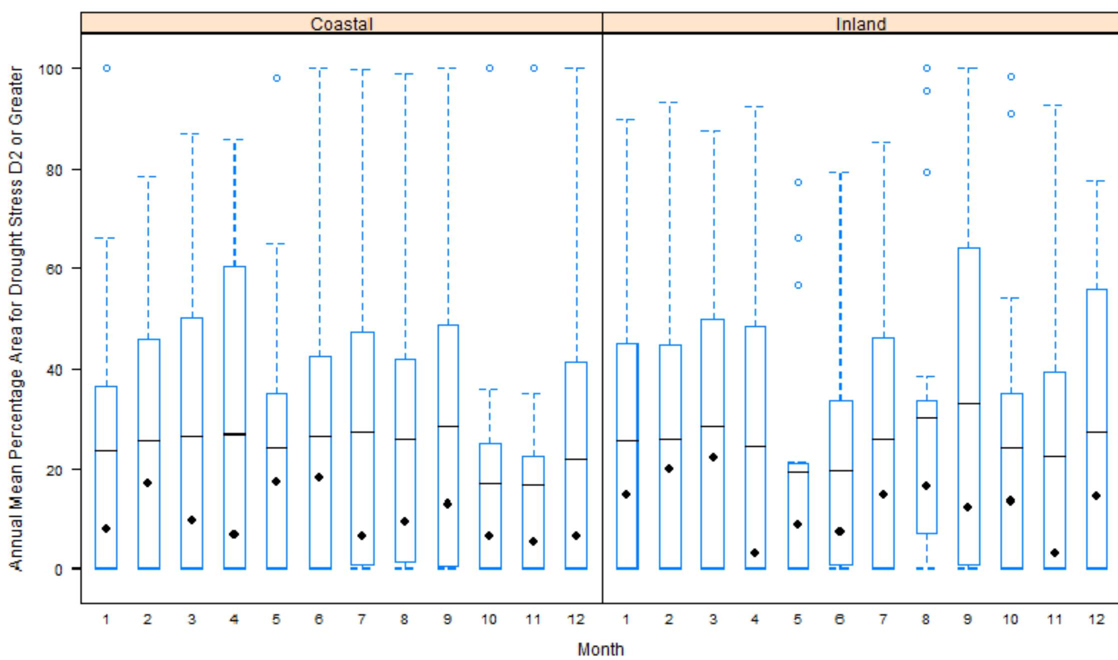
F - 3. Annual Mean Percentage Area for Drought Stress D2



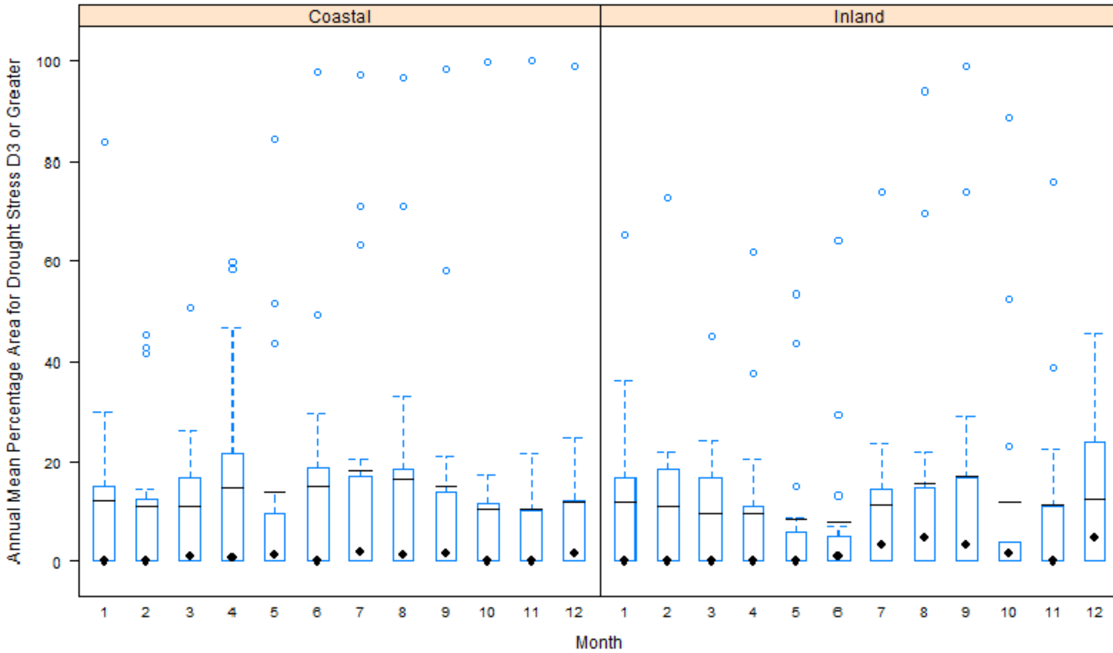
F- 4. Annual Mean Percentage Area for Drought Stress D3



F - 5. Annual Mean Percentage Area for Drought Stress D4



F - 6. Annual Mean Percentage Area for Drought Stress GE2



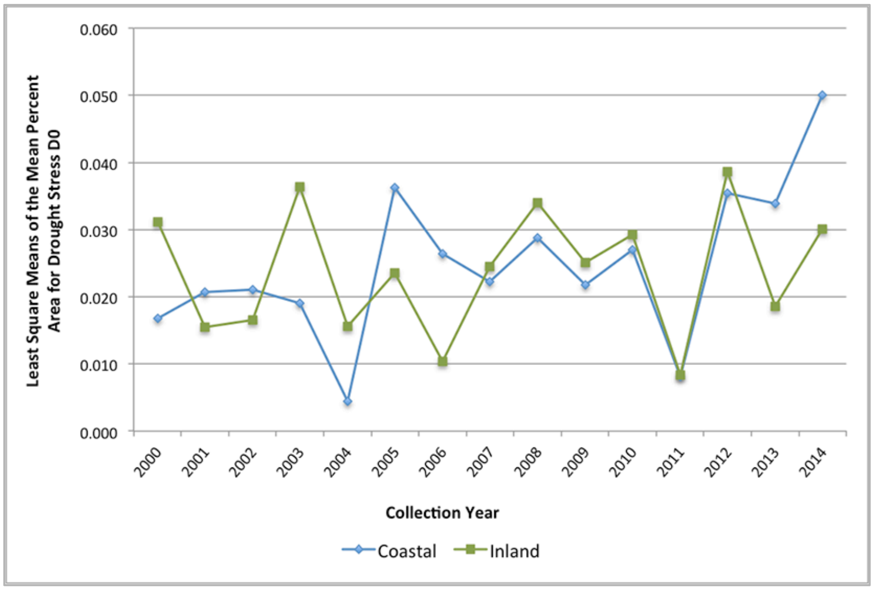
F - 7. Annual Mean Percentage Area for Drought Stress GE3

Month	Zone	Annual Mean Percent Area													
		D0 Mean	D0 Median	D1 Mean	D1 Median	D2 Mean	D2 Median	D3 Mean	D3 Median	D4 Mean	D4 Median	GE2 Mean	GE2 Median	GE3 Mean	GE3 Median
1	Coastal	1.492911	0.9973	1.444243	0.81472	1.2950467	0.9203	0.834462	0	0.5008133	0	23.67413	8.003	12.193133	0
2	Coastal	1.14766	0.71765	1.551072	1.91736	1.6343733	1.4669	0.839612	0	0.3473467	0	25.7316	17.066	11.042933	0
3	Coastal	1.388854	1.13231	1.470976	1.22248	1.6499733	0.6842	0.7046107	0.05957	0.5062533	0	26.68247	9.753	11.185533	0.834
4	Coastal	1.316196	0.79438	1.358263	0.57673	1.29724	0.5825	0.7756713	0.04798	0.7447933	0	26.91553	6.808	14.500467	0.595
5	Coastal	2.295406	2.77346	2.22204	2.25318	1.0672733	0.8374	0.6449687	0.09922	0.8019	0	24.21073	17.317	13.872867	1.357
6	Coastal	2.039642	1.94258	2.330633	1.56895	1.23056	0.5254	0.7277867	0.00879	0.8516067	0	26.53273	18.221	14.966467	0.105
7	Coastal	1.831025	1.5243	1.781256	1.61391	0.92712	0.2762	0.802938	0.15605	1.15914	0	27.06353	6.432	18.1804	1.899
8	Coastal	1.472863	1.40469	1.876573	1.68101	1.0161067	0.5372	0.6171933	0.10313	1.1538467	0	25.93547	9.399	16.381933	1.234
9	Coastal	1.325621	1.22545	1.155463	0.91784	1.45508	0.2626	0.6414533	0.12453	0.9857933	0	28.20367	12.938	14.954	1.52
10	Coastal	1.552421	1.14341	1.871218	1.23153	0.7161	0.3042	0.36681	0.00048	0.7736667	0	17.04433	6.411	10.491933	0.005
11	Coastal	1.863599	1.5762	1.515197	0.98206	0.6620267	0.4355	0.419606	0	0.7250333	0	16.5266	5.326	10.609	0
12	Coastal	1.870829	1.59363	1.138533	0.65854	1.1191133	0.4471	0.6838433	0.12454	0.61512	0	21.97553	6.495	11.918667	1.505
1	Inland	1.795823	1.34775	1.797905	2.40079	1.5860267	1.0192	1.022246	0	0.37388	0	25.81527	14.704	11.990267	0
2	Inland	1.725691	1.3701	1.811056	1.93785	1.6945667	1.0126	0.9910107	0	0.2730467	0	26.05987	19.981	11.062667	0
3	Inland	1.633615	1.3607	1.571537	1.7314	2.11306	1.5783	0.9788707	0	0.1339333	0	28.36927	22.225	9.719733	0
4	Inland	1.857899	1.57578	1.086463	0.86552	1.6754067	0.2852	0.9654507	0.00877	0.1519933	0	24.53093	3	9.782067	0.095
5	Inland	2.50047	1.67377	1.96084	1.4903	1.2257267	0.6427	0.664698	0.00135	0.2708	0	19.2504	8.876	8.447533	0.011
6	Inland	2.48628	2.49267	2.161247	2.55923	1.39668	0.7798	0.5514913	0.10859	0.3532467	0	19.6406	7.351	8.0094	1.037
7	Inland	2.278117	2.66008	1.95486	1.91044	1.7381667	0.59	0.86707	0.34288	0.46768	0	25.99793	14.505	11.362467	3.119
8	Inland	2.180949	2.39252	2.160569	2.04018	1.7049533	1.5039	0.9517047	0.52311	0.8828267	0	30.0034	16.467	15.491467	4.803
9	Inland	1.787085	1.81022	1.187421	0.72316	1.90946	0.5598	1.1160167	0.47321	0.8840867	0	33.007	12.288	16.817533	3.391
10	Inland	1.770537	1.56201	1.720177	0.46307	1.4570933	0.9551	0.7706153	0.13734	0.6709	0	24.38547	13.52	11.8826	1.476
11	Inland	1.85899	1.97349	1.609427	0.88454	1.2900533	0.2667	0.9447333	0.01598	0.4458467	0	22.59973	2.837	11.4904	0.136
12	Inland	1.393996	0.66229	1.725031	1.7728	1.6791867	0.4501	1.1633647	0.43489	0.3512867	0	27.1616	14.347	12.596067	4.6

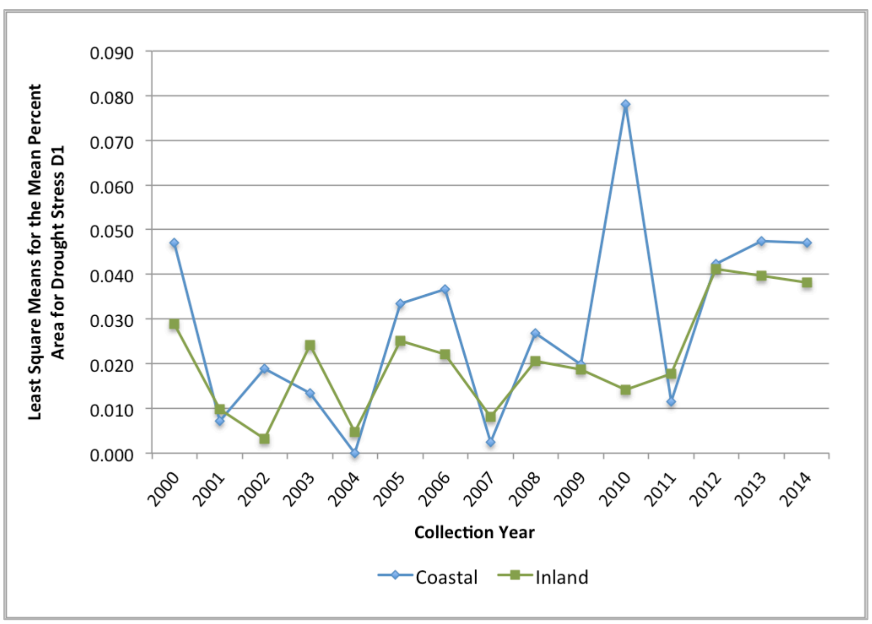
F - 8. Mean and Median of Annual Mean Percentage Area for All Drought Stress Categories, by Month, for the Coastal and Inland Zone

APPENDIX G

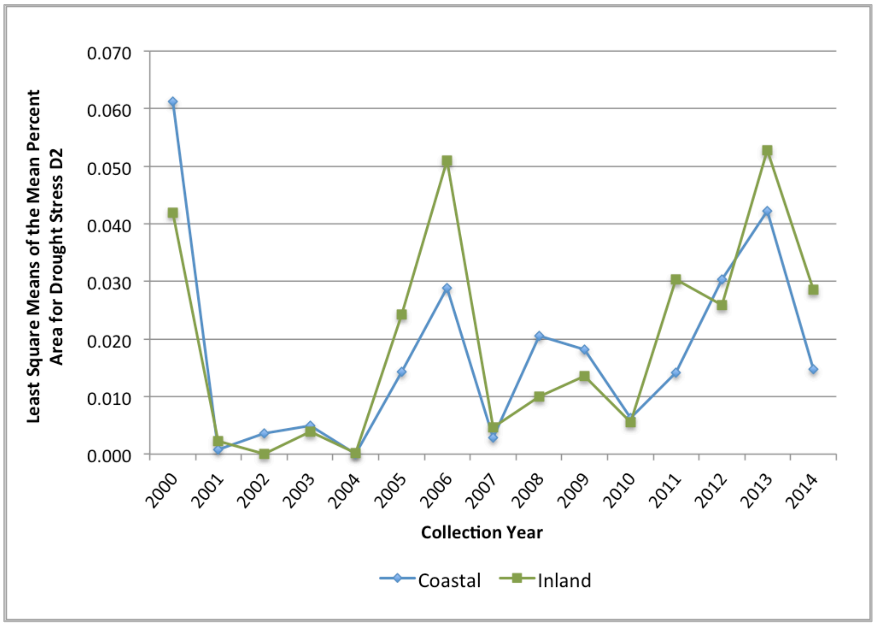
LEAST SQUARE MEANS OF THE MEAN PERCENT AREA FOR DROUGHT STRESS CATEGORIES



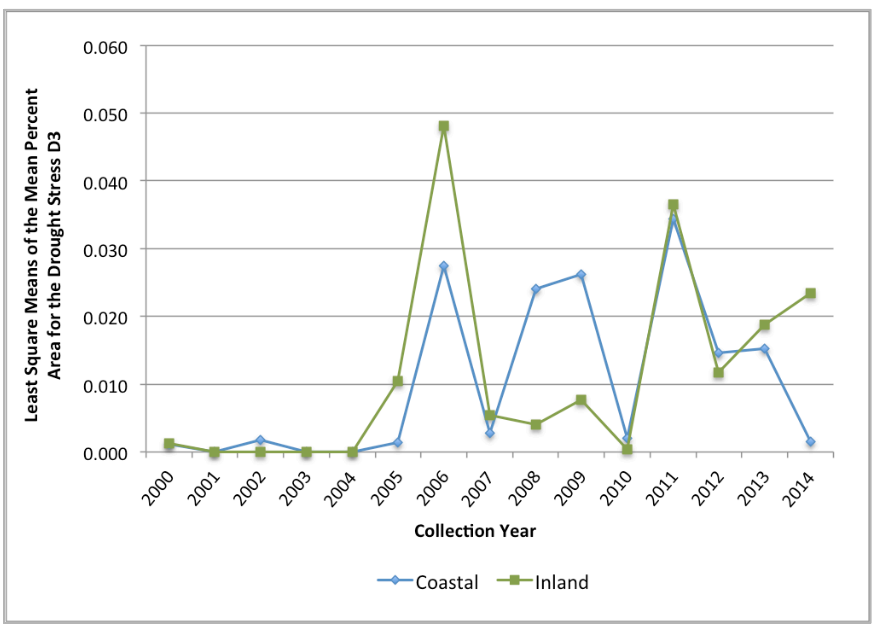
G - 1. Least Square Means of the Mean Percent Area for Drought Stress D0



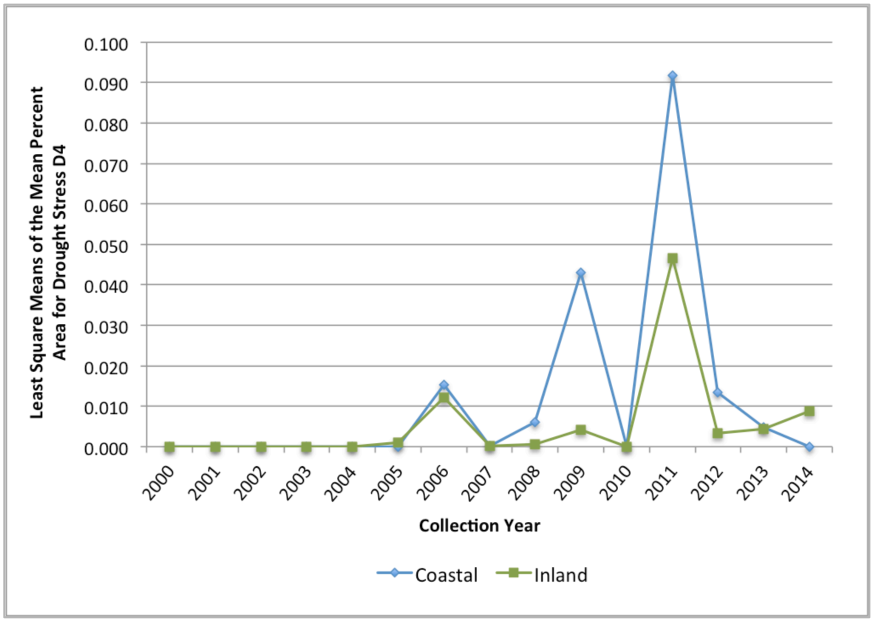
G - 2. Least Square Means of the Mean Percent Area for Drought Stress D1



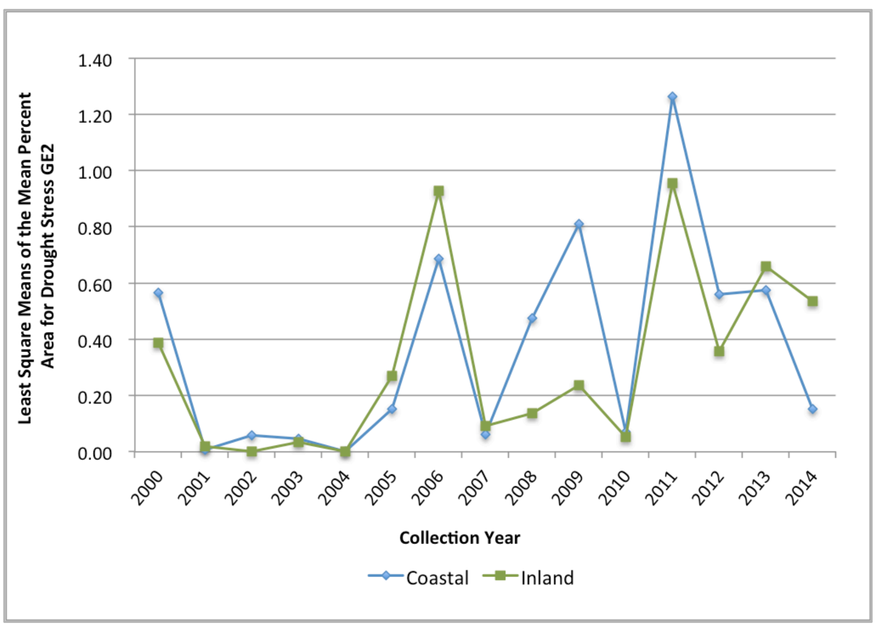
G - 3. Least Square Means of the Mean Percent Area for Drought Stress D2



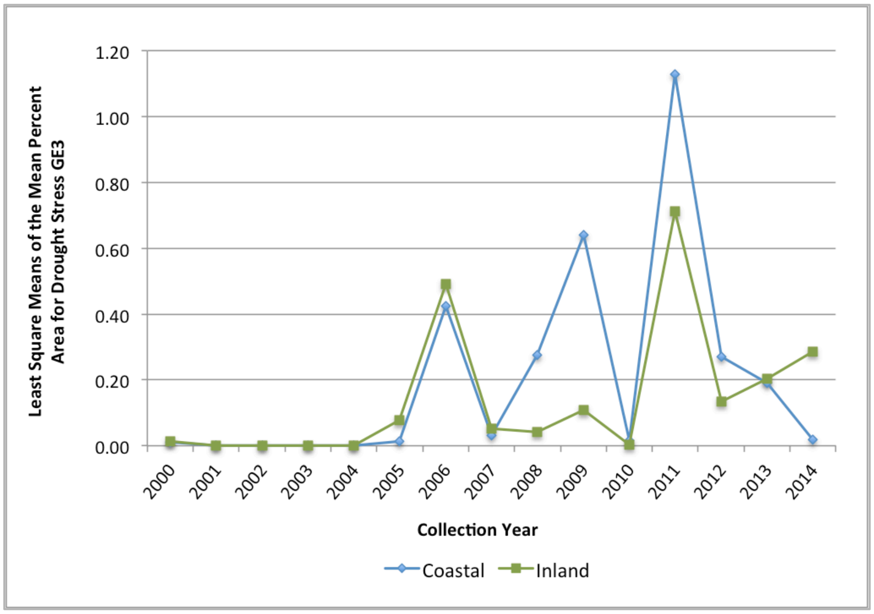
G - 4. Least Square Means of the Mean Percent Area for Drought Stress D3



G - 5. Least Square Means of the Mean Percent Area for Drought Stress D4



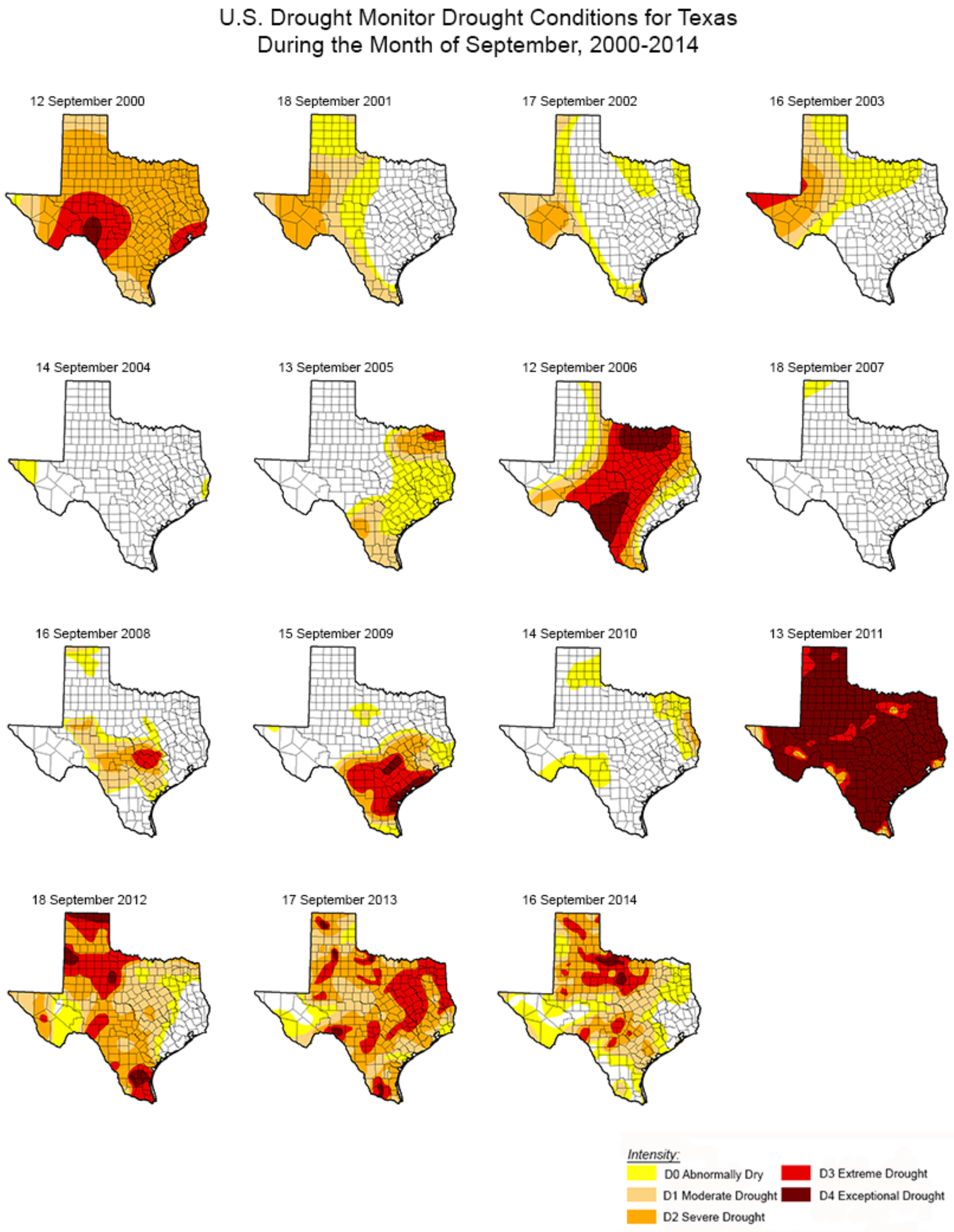
G - 6. Least Square Means of the Mean Percent Area for Drought Stress GE2



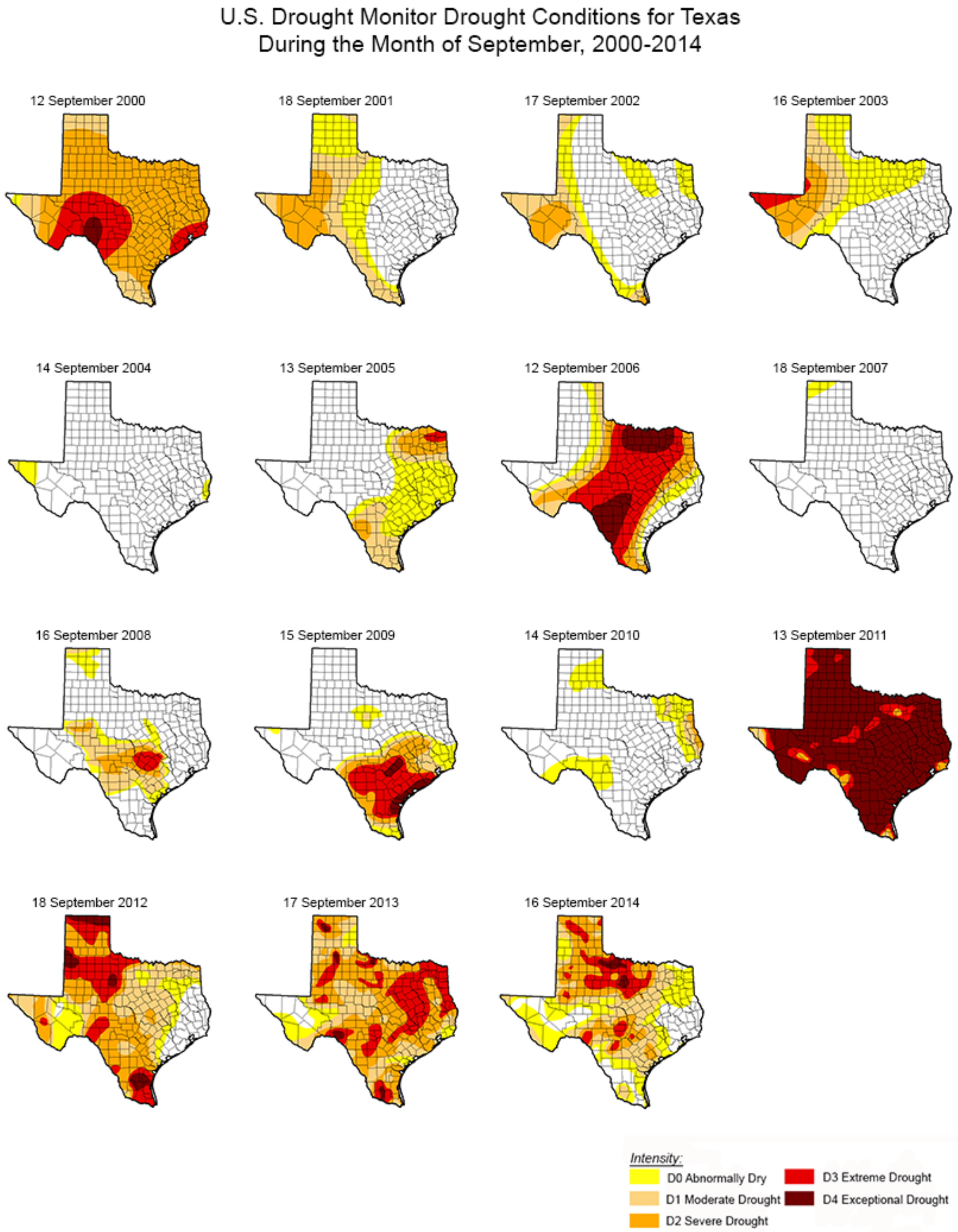
G - 7. Least Square Means of the Mean Percent Area for Drought Stress GE3

APPENDIX H

USDM MAPS, FEBRUARY AND SEPTMEBER, 2000 – 2014



H - 1. U.S. Drought Monitor Drought Stress Conditions for Texas During the Month of February, 2000 – 2014



H - 2. U.S. Drought Monitor Drought Stress Conditions for Texas During the Month of February, 2000 – 2014

APPENDIX I

COUNTY CLUSTERS

Coastal Zone								
County "Cluster" Center (Primary County)	**Counties Adjacent to the Center (Primary County) in County "Clusters"**							
Atascosa	Bexar	Frio	Karnes	La Salle	Live Oak	McMullen	Medina	Wilson
Austin	Colorado	Fayette	Fort Bend	Waller	Washington	Wharton		
Bastrop	Caldwell	Fayette	Gonzales	Lee	Travis	Williamson		
Bee	Goliad	Karnes	Live Oak	Refugio	San Patricio			
Brazos	Burleson	Grimes	Leon	Madison	Robertson	Washington		
Burleson	Brazos	Lee	Milam	Robertson	Washington			
Caldwell	Bastrop	Fayette	Gonzales	Guadalupe	Hays	Travis		
Colorado	Austin	Fayette	Jackson	Lavaca	Wharton			
DeWitt	Goliad	Gonzales	Karnes	Lavaca	Victoria			
Fayette	Austin	Bastrop	Caldwell	Colorado	Gonzales	Lavaca	Lee	Washington
Gonzales	Caldwell	DeWitt	Fayette	Guadalupe	Karnes	Lavaca	Wilson	
Grimes	Brazos	Madison	Montgomery	Walker	Waller	Washington		
Guadalupe	Bexar	Caldwell	Comal	Gonzales	Hays	Wilson		
Jim Wells	Brooks	Duval	Kleberg	Live Oak	McMullen	Nueces	San Patricio	
Lavaca	Colorado	DeWitt	Fayette	Gonzales	Jackson	Victoria	Wharton	
Lee	Bastrop	Burleson	Fayette	Milam	Washington	Williamson		
Washington	Austin	Brazos	Burleson	Fayette	Grimes	Lee	Waller	

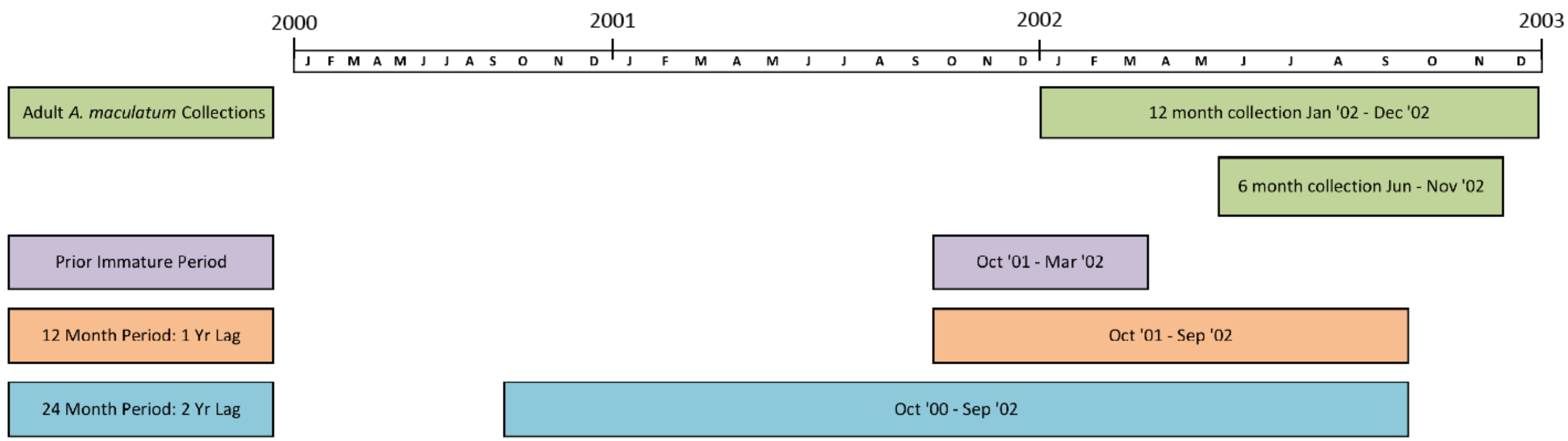
I - 1. County "Clusters" for Drought Stress Level Analysis in the Coastal Zone

Inland Zone								
County "Cluster" Center (Primary County)	**Counties Adjacent to the Center (Primary County) in County "Clusters"**							
Archer	Baylor	Clay	Jack	Throckmorton	Young	Wichita	Wilbarger	
Bosque	Coryell	Erath	Hamilton	Hill	Johnson	McLennan	Sommervell	
Cherokee	Anderson	Angelina	Henderson	Houston	Nacogdoches	Rusk	Smith	
Comanche	Brown	Eastland	Erath	Hamilton	Mills			
Coryell	Bell	Bosque	Hamilton	Lampasas	McLennan			
Erath	Bosque	Comanche	Eastland	Hamilton	Hood	Palo Pinto	Sommervell	
Gillespie	Blanco	Kendall	Kerr	Kimble	Llano	Mason		
Gregg	Harrison	Rusk	Smith	Upshur				
Hamilton	Bosque	Comanche	Coryell	Erath	Lampasas	Mills		
Henderson	Anderson	Cherokee	Ellis	Freestone	Kaufman	Navarro	Smith	Van Zandt
Hill	Bosque	Ellis	Johnson	Limestone	McLennan	Navarro		
Hopkins	Delta	Franklin	Hunt	Rains	Red River	Wood		
Lamar	Delta	Fannin	Franklin	Hopkins	Red River			
Lampasas	Bell	Burnet	Coryell	Hamilton	Mills	San Saba		
Limestone	Falls	Freestone	Hill	Leon	McLennan	Navarro	Robertson	
McLennan	Bell	Bosque	Coryell	Falls	Hill	Limestone		
Milam	Bell	Burleson	Falls	Lee	Robertson	Williamson		
Montague	Clay	Cooke	Jack	Wise				
Nacogdoches	Angelina	Cherokee	Rusk	San Augustine	Shelby			
Palo Pinto	Eastland	Erath	Hood	Jack	Parker	Stephens	Young	
Panola	Harrison	Rusk	Shelby					
Rains	Hopkins	Hunt	Lamar	Smith	Van Zandt	Wise		
Robertson	Brazos	Burleson	Falls	Leon	Limestone	Madison	Milam	
Rusk	Cherokee	Gregg	Harrison	Nacogdoches	Panola	Shelby	Smith	
San Saba	Brown	Burnet	Lampasas	Llano	Mason	McCulloch	Mills	
Shelby	Nacogdoches	Panola	Rusk	Sabine	San Augustine			
Van Zandt	Henderson	Hunt	Kaufman	Rains	Smith	Wood		
Wise	Cooke	Denton	Jack	Montague	Parker	Tarrant		
Wood	Camp	Franklin	Hopkins	Rains	Smith	Upshur	Van Zandt	

I - 2. County "Clusters" for Drought Stress Level Analysis in the Inland Zone

APPENDIX J

CLUSTER ANALYSIS TIMELINE AND DROUGHT STRESS FACTOR EXAMPLE



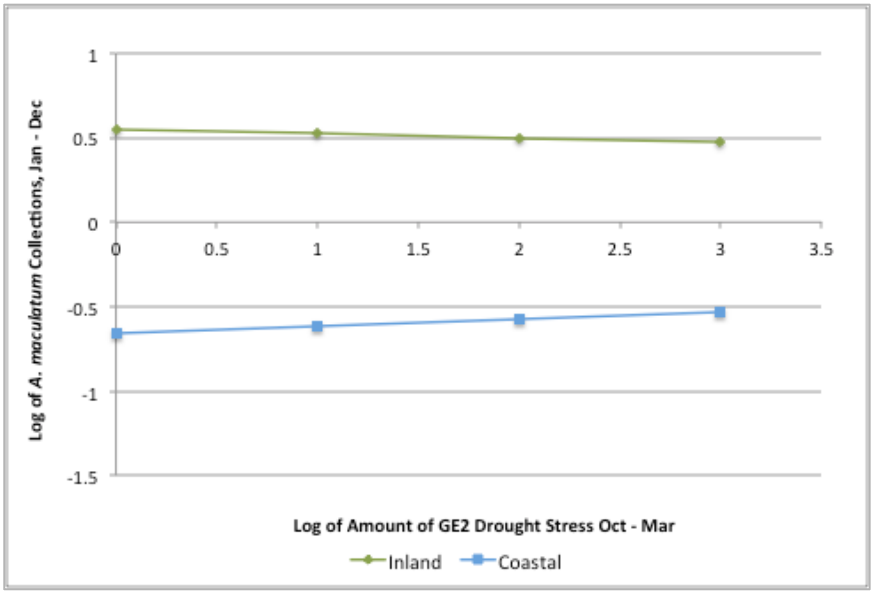
J - 1. Visual Explanation for the Tick Collections and Lag Period for Drought Stress Analysis

Primary County	Collection Year	12-month Tick Collection	6-month Tick Collection	2 Yr Lag Drought	Drought Stress Factor
Primary_Co	Year	logTicks	logTicks6_11	logGE2_2YL	logGE2_Factor
Archer	2000	0.69315	0.69315	1.60908	Medium
Archer	2001	0.00000	0.00000	1.90298	Medium
Archer	2002	0.00000	0.00000	1.54900	Medium
Archer	2003	0.00000	0.00000	1.01541	Low
Archer	2004	0.00000	0.00000	1.20359	Low
Archer	2005	0.00000	0.00000	0.98802	Low
Archer	2006	0.00000	0.00000	2.81986	Medium
Archer	2007	0.00000	0.00000	2.91242	Medium
Archer	2008	0.00000	0.00000	1.32409	Low
Archer	2009	0.00000	0.00000	2.22324	Medium
Archer	2010	0.00000	0.00000	2.22132	Medium
Archer	2011	0.00000	0.00000	2.72721	Medium
Archer	2012	0.00000	0.00000	3.44487	High
Archer	2013	0.00000	0.00000	3.70065	High
Archer	2014	0.69315	0.69315	3.86414	High

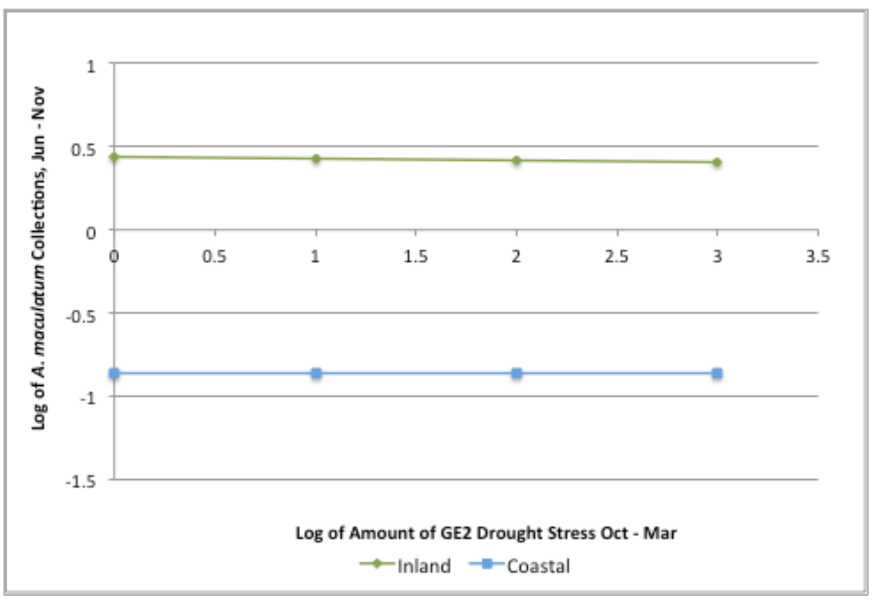
J - 2. Example of Drought Stress Factor for GE2 Drought Stress in Archer County

APPENDIX K

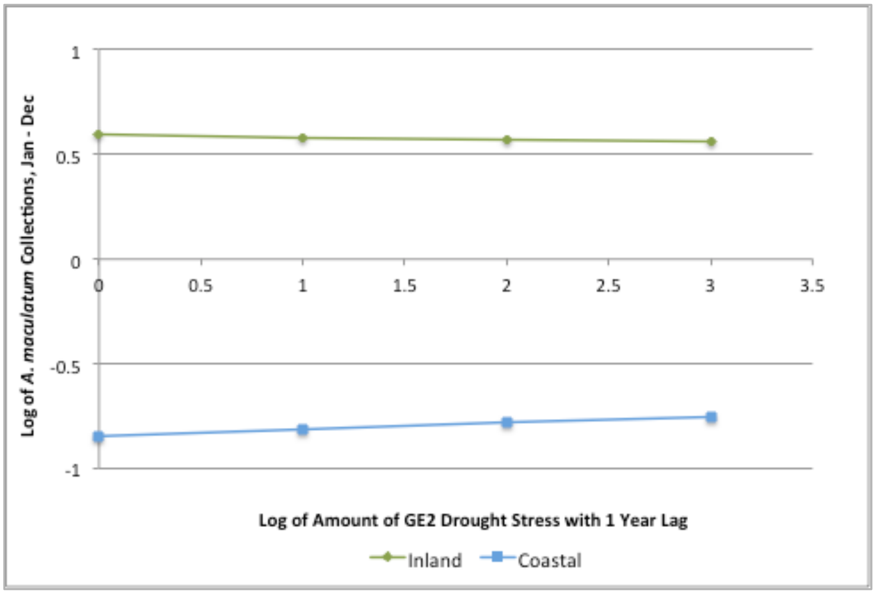
SLOPE-INTERCEPT FORM PLOTS FOR TICK COLLECTIONS AND GE2 DROUGHT STRESS



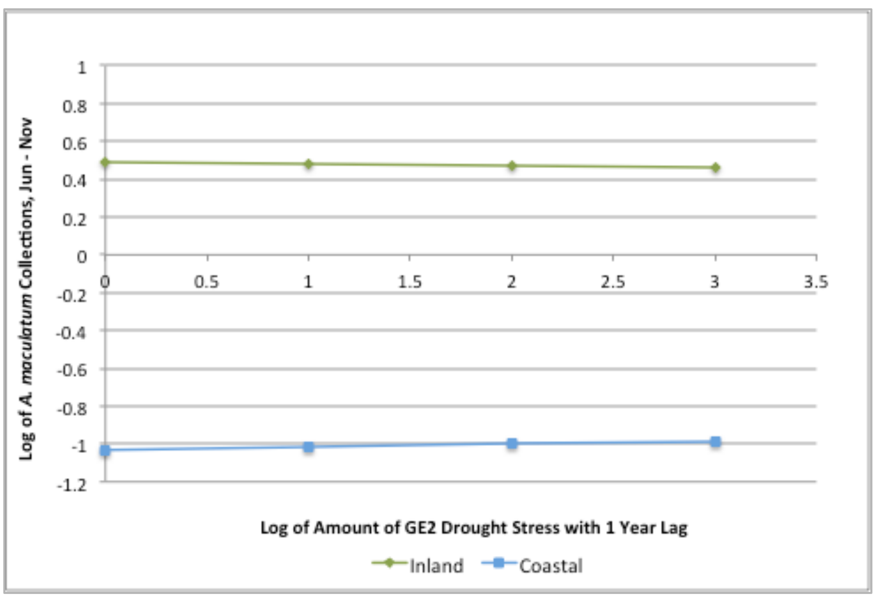
K - 1. Repeated Measures Analysis Slope-Intercept Form Results for Tick Collections, Jan – Dec and GE2 Drought Stress, Oct – Mar



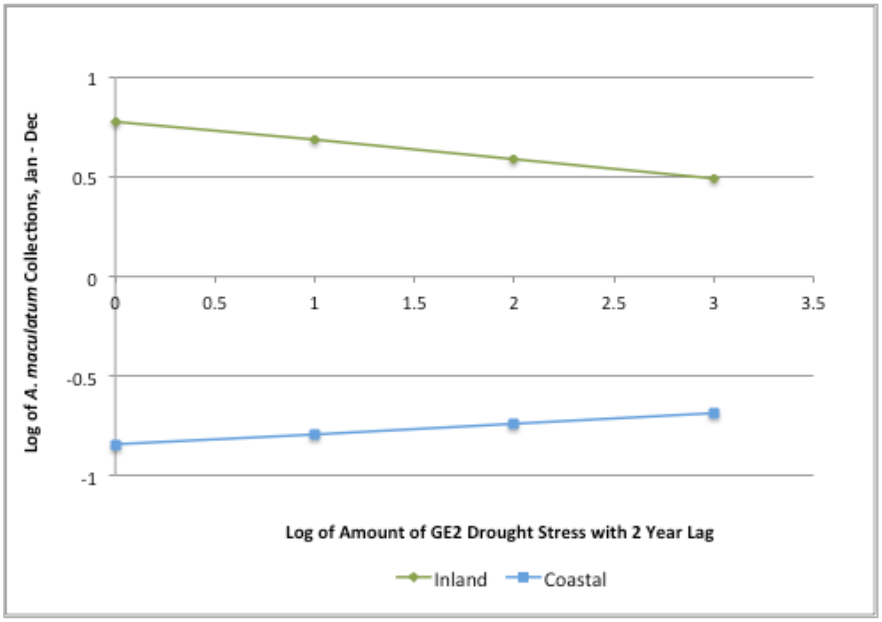
K - 2. Repeated Measures Analysis Slope-Intercept Form Results for Tick Collections, Jun - Nov and GE2 Drought Stress, Oct – Mar



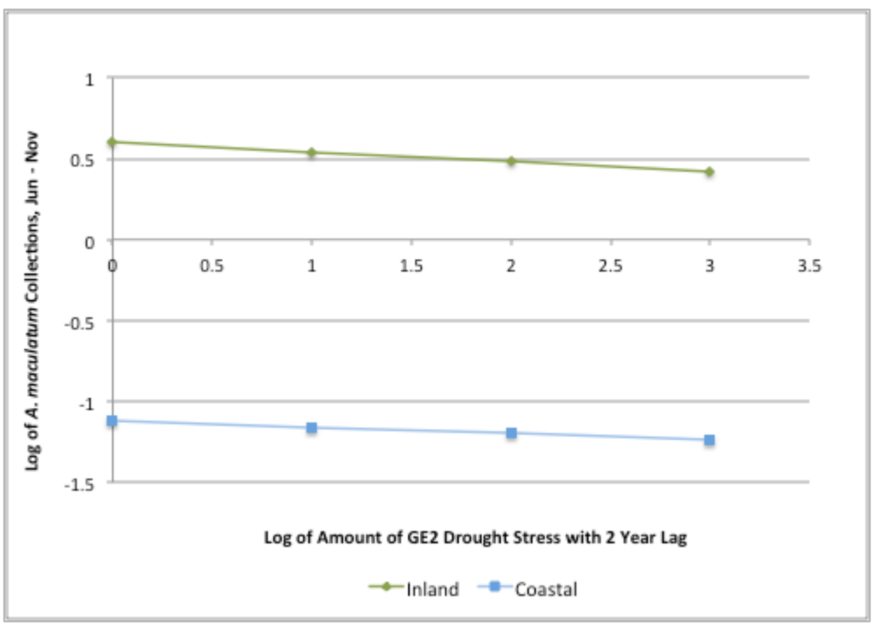
K - 3. Repeated Measures Analysis Slope-Intercept Form Results for Tick Collections, Jan – Dec and GE2 Drought Stress, 1-year lag



K - 4. Repeated Measures Analysis Slope-Intercept Form Results for Tick Collections, Jun - Nov and GE2 Drought Stress, 1-year lag



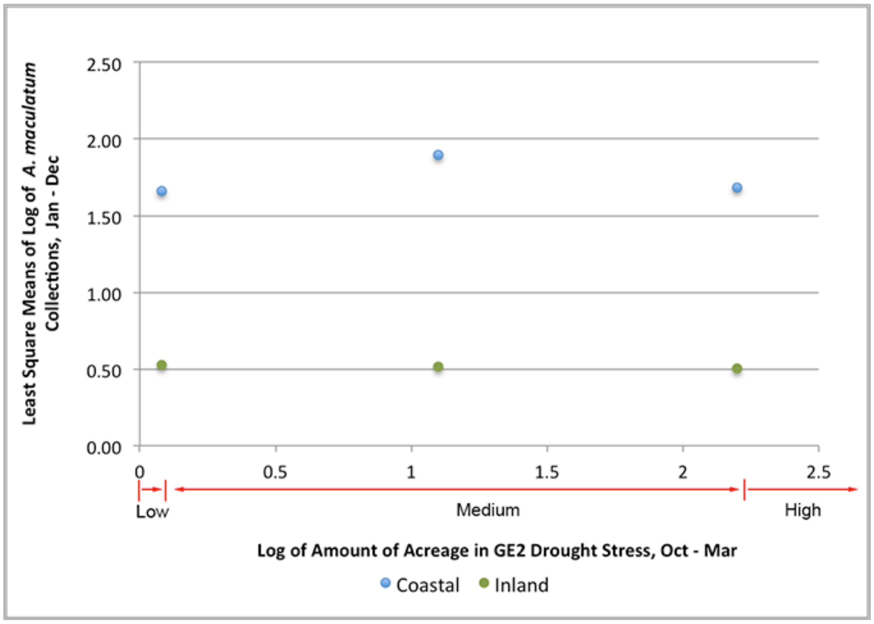
K - 5. Repeated Measures Analysis Slope-Intercept Form Results for Tick Collections, Jan – Dec and GE2 Drought Stress, 2-year lag



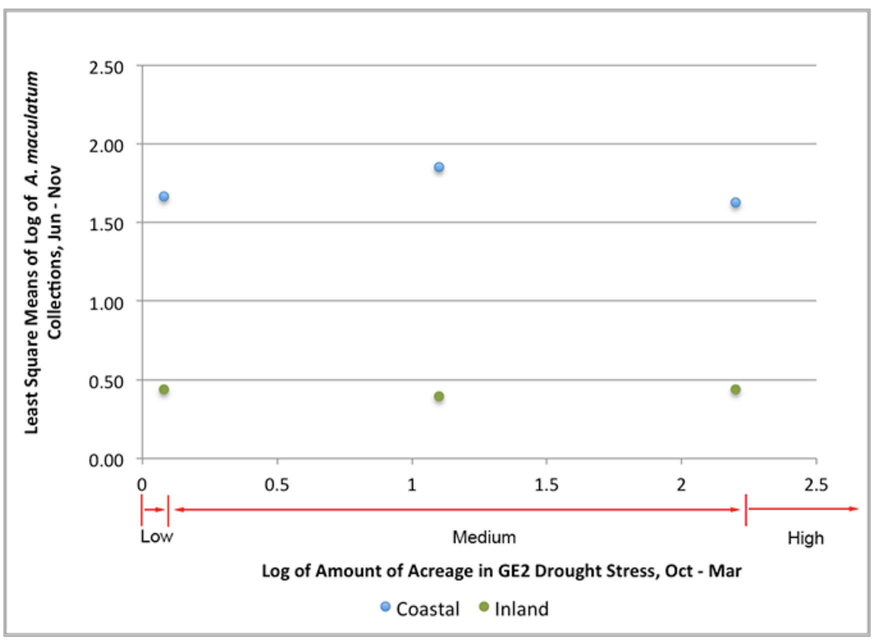
K - 6. Repeated Measures Analysis Slope-Intercept Form Results for Tick Collections, Jun - Nov and GE2 Drought Stress, 2-year lag

APPENDIX L

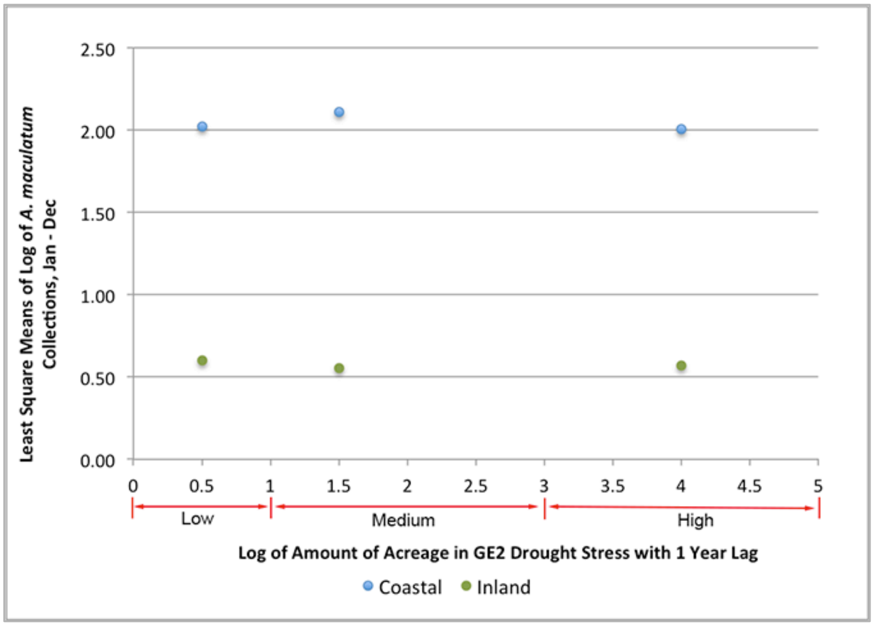
FACTOR PLOTS FOR TICK COLLECTIONS AND GE2 DROUGHT FACTORS



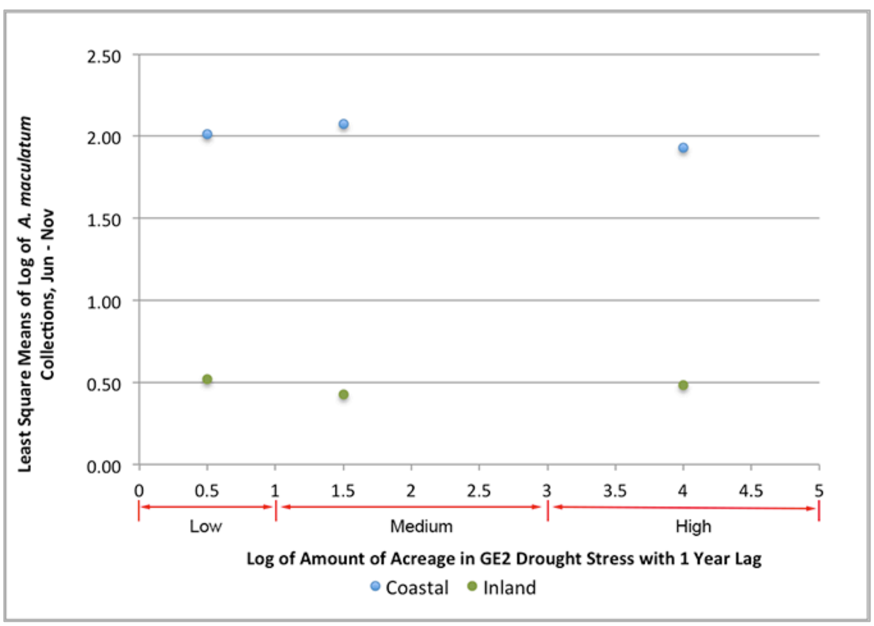
L - 1. Repeated Measures Analysis Least Square Means Results for Tick Collections, Jan – Dec and GE2 Drought Stress Factors (Low, Medium and High), Oct – Mar



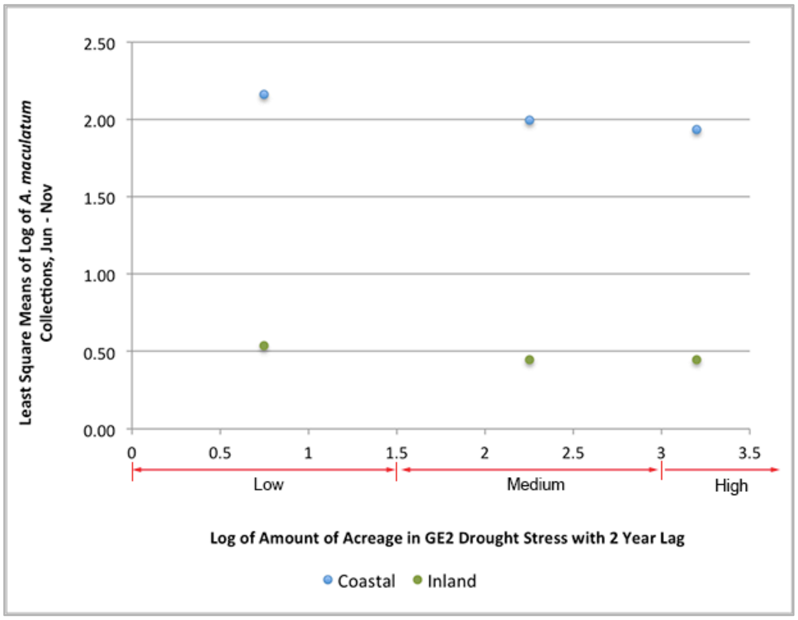
L - 2. Repeated Measures Analysis Least Square Means Results for Tick Collections, Jun - Nov and GE2 Drought Stress Factors (Low, Medium and High), Oct – Mar



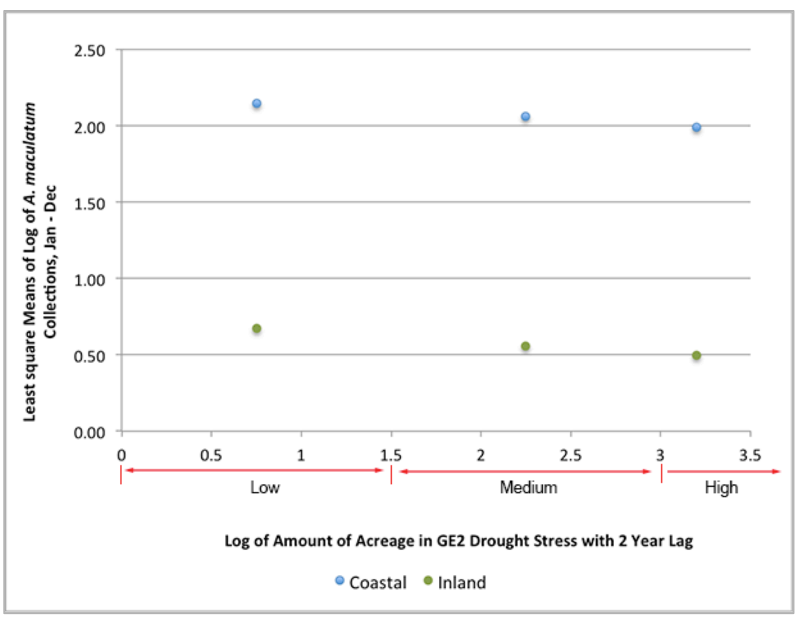
L - 3. Repeated Measures Analysis Least Square Means Results for Tick Collections, Jun - Nov and GE2 Drought Stress Factors (Low, Medium and High), Oct – Mar



L - 4. Repeated Measures Analysis Least Square Means Results for Tick Collections, Jun - Nov and GE2 Drought Stress Factors (Low, Medium and High), 1-Year Lag



L - 5. Repeated Measures Analysis Least Square Means Results for Tick Collections, Jan – Dec and GE2 Drought Stress Factors (Low, Medium and High), 2-Year Lag



L - 6. Repeated Measures Analysis Least Square Means Results for Tick Collections, Jun - Nov and GE2 Drought Stress Factors (Low, Medium and High), 2-Year Lag